PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Lois WEISMAN

Serial No.: 10/791,219

Filed: March 2, 2004

For: INTRACELLULAR SIGNALING PATHWAYS IN DIABETIC SUBJECTS

Group Art Unit: 1653

Examiner: Samuel Liu

Atty. Dkt. No.: IOWA:048US/SLH

Confirmation No.: 3887

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May 5, 2008
Date

Steven L. Highlander

APPEAL BRIEF

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APPEAL BRIEF

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-01450

Dear Sir:

This Appeal Brief is filed in response to the final Office Action mailed on October 31, 2007, and the Advisory Action mailed on January 7, 2008. Appellant's brief is due on May 5, 2008 (since May 3, 2008 falls on a Saturday), by virtue of the Notice of Appeal filed on December 3, 2008, and the accompanying Petition for Extension of Time (three months) and payment of fees. Also included herewith is the fee for the brief. No other fees are believed due in connection with this filing; however, should appellant's payment be missing or deficient, or should any fees be due, the Commissioner is authorized to debit Fulbright & Jaworski L.L.P. Deposit Acct. No. 50-1212/IOWA:048US/SLH.

I. Real Party In Interest

The real party in interest is the assignee, the University of Iowa Research Foundation, Iowa City, IA.

II. Related Appeals and Interferences

There are no related appeals or interferences.

III. Status of the Claims

Claims 1-60 were filed with the original application, and claims 1-17, 20-23 and 27-32 have been canceled. Claims 33-60 stand withdrawn pursuant to a restriction requirement, claim 18 is allowed, and claim 19 is objected to. Claims 24-26 are under consideration, stand rejected and are the subject of this appeal. A copy of the appealed claims is attached as Appendix A1. A copy of the appealed claims with proposed amendments is attached as Appendix A2.

IV. Status of the Amendments

The amendments offered after mailing of the final Office Action were denied entry in the Advisory Action dated January 7, 2008. A second amendment is being filed concurrent with this brief (Exhibit 2), entry of which is hereby requested.

V. Summary of the Claimed Subject Matter

Independent claim 24 is supported in the specification at page 4, lines 3-11.

VI. Ground of Rejection to be Reviewed on Appeal

A. Are claims 24-26 lacking adequate written description in the specification?

B. Are claims 24-26 lacking an enabling disclosure in the specification?

C. Are claims 24-26 indefinite over use of the term about?

D. Are claims 24-26 anticipated by Robert *et al.* (Exhibit 1)?

VII. Argument

A. Standard of Review

Findings of fact and conclusions of law by the U.S. Patent and Trademark Office must be made in accordance with the Administrative Procedure Act, 5 U.S.C. §706(A), (E), 1994. *Dickinson v. Zurko*, 527 U.S. 150, 158 (1999). Moreover, the Federal Circuit has held that findings of fact by the Board of Patent Appeals and Interferences must be supported by "substantial evidence" within the record. *In re Gartside*, 203 F.3d 1305, 1315 (Fed. Cir. 2000). In *In re Gartside*, the Federal Circuit stated that "the 'substantial evidence' standard asks whether a reasonable fact finder could have arrived at the agency's decision." *Id.* at 1312. Accordingly, it necessarily follows that an examiner's position on appeal must be supported by "substantial evidence" within the record in order to be upheld by the Board of Patent Appeals and Interferences.

B. Rejections Under 35 U.S.C. §112, First Paragraph

i. Written Description.

Claims 24-26 stand rejected as lacking an adequate written description. In the first Office Action, the examiner set forth 5 factors for analyzing the written description requirement. Of these, level of skill in the art and method of making clearly mitigate ***in favor*** of written description, as the skill is admittedly high, and making peptides of a known sequence is trivial.

With regard to physcial and/or chemical properties, appellant submits that one use of the claims oligopeptides is to produce antibodies, which in turn can be used to assay for the full-length protein. Though not a universal property of each and every polypeptides, ***in the absence of any evidence to the contrary, appellant submits that one of skill in the art would presume***

that many if not most peptides would be useful in this context. In the final Office Action, the examiner acknowledged this but then attempted to rebut this line of argument by citing to Prescott *et al.* (Exhibit 3). This citation is highly misleading and completely irrelevant to appellant's argument ***as the Prescott reference discusses immunotherapy, not the simple production of antibodies.*** To even attempt to support the rejection in this way belies a fundamental misunderstanding of the invention, or even worse, a complete lack of foundational support in the literature for this rejection. Moreover, the examiner's continued digressions into "inhibitory activity" are irrelevant when determining written description.

With regard to functional characteristics/partial structure, appellant submits that while the number of possible oligopeptides falling within the scope of the claim is large, it is a straightforward matter to identify each of these. Indeed, appellant could easily have generated a sequence listing with each and every possible peptide using a computer program and filled the Patent Office with hundreds of pages of a sequence listing. However, since it would immediately be evident to any skilled artisan up a reading of the specification what peptides were envisioned, there would be no reason so submit such a listing. And again, one can easily determine which oligopeptides can generate antibodies. In the absence of some ***meaningful*** reason to doubt that property, one would also assume that a given peptide ***would*** have such activity.

In sum, while properly citing factors that should be applied to a written description analysis, the examiner then fails to actually ***follow*** the results of the analysis, and instead focuses on isolated factors that are incorrectly assessed or fail to address the nature of the claimed invention. Reversal of the rejection is again therefore respectfully requested.

ii. Enablement.

Claims 24-26 stand rejected as lacking an enabling disclosure. The examiner has made proper reference to the factors under consideration when analyzing enablement under §112, first paragraph, but the examiner has failed to use valid facts and has come to an incorrect conclusion regarding enablement.

Again, as above, appellant readily admits that the genus of claimed oligopeptides is large. However, it simply cannot be said that the skilled artisan cannot correlate function of the ***genus*** of oligopeptides of SEQ ID NO:3 with the biological activity of raising antibodies. As noted in the application at page 26, one can use peptides to produce antibodies which can then be used diagnostic applications. And while it is true that not ***every*** oligogpeptide will produce antibodies that cross-react with the native Vac14 sequence, one would expect many (in fact the ***majority***) of such oligopeptides to elicit at least a polyclonal response that would react to some extent with a denatured form of SEQ ID NO:3. The examiner's reference to Prsecott *et al.*, as discussed above, is ***completely*** off the mark as the reference deals with allergy vaccines, not the simple production of antibodies. Generation of antibodies is sufficient to enable the claimed peptides generally, and the possible inclusion of a few inoperative species within the scope of a claim does not necessarily render a claim nonenabled. See MPEP §2164.08. "The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art." Citing *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984). Even a large number of operable embodiments does not always render a claim broader than the enabled scope when undue experimentation is not involved in determining which embodiments are operable. See *In re Angstadt*, 537 F.2d 498, 502-503, 190

USPQ 214, 218 (CCPA 1976). And just as with the written description analysis, the attack on "inhibitory activity" of the peptides is completely irrelevant.

Here, there remains no meaningful challenge to appellant's argument that raising antibodies or antiserum against a given peptide is trivial from a scientific standpoint, as is the testing of such compositions for reactivity with Vac14. As such, there can be no real question as to the enablement of claims 24-26. Reversal of the rejection is therefore respectfully requested.

C. Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 24-26 stand newly rejected under the second paragraph of §112 for use of the term "about" in claim 24. Appellant traverses, but in the interest of advancing the prosecution, an amendment was offered that deleted "about." This amendment was denied entry by the Advisosy Action as allegedly not reducing the issues on appeal. Appellant again states the willingness to amend the claims to address the rejection, and have submitted another amendment which is hoped to overcome the examiner's concern that led to its non-entry (Exhibit 2). Reversal of the rejection is therefore respectfully requested.

D. Rejection Under 35 U.S.C. §102(b)

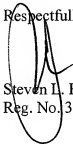
Claims 24-26 stand rejected as anticipated by Robert *et al.* (Exhibit 1), which is said to disclose an oligopeptide of 10 residues with 100% identity to a portion of SEQ ID NO:3. Appellant's last submission proposed an amendment of the claims to recite a minimum length of 15 residues. This amendment was denied entry by the Advisory Action as allegedly not reducing the issues on appeal. Though not explicitly stated in the Advisory Action, the non-entry may have been due to the failure to include 15 as the minimum contiguous portion of SEQ ID NO:3.

Appellant is submitting herewith a second amendment to address the rejection (Exhibit 2). Reversal of the rejection is therefore respectfully requested.

E. Conclusion

In light of the foregoing, appellant respectfully submits that all pending claims are non-obvious under 35 U.S.C. §103. Therefore, it is respectfully requested that the Board reverse each of the pending rejections.

Respectfully submitted,



Date: May 5, 2008

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VIII(1). APPENDIX A1 – APPEALED CLAIMS

24. An oligopeptide of between 10 and about 30 residues, said oligopeptide comprising at least 10 consecutive residues of SEQ ID NO:3.

25. The oligopeptide of claim 24, wherein said oligopeptide is 10, 15, 20, 25, or 30 residues in length.

26. The oligopeptide of claim 24, wherein the number of consecutive residues is 10, 15, 20, 25, or 30.

VIII(2). APPENDIX A2 – APPEALED CLAIMS WITH PROPOSED AMENDMENTS

24. (Currently amended) An oligopeptide of between **[[**10**]]**15 and ~~about~~ 30 residues, said oligopeptide comprising at least **[[**10**]]**15 consecutive residues of SEQ ID NO:3.

25. (Currently amended) The oligopeptide of claim 24, wherein said oligopeptide is **[[**10, **]]**15, 20, 25, or 30 residues in length.

26. (Currently amended) The oligopeptide of claim 24, wherein the number of consecutive residues is **[[**10, **]]**15, 20, 25, or 30.

IX. APPENDIX B – EVIDENCE CITED

Exhibit 1 – Roberts *et al.*

Exhibit 2 – Concurrently filed amendment under 37 C.F.R. §1.116

Exhibit 3 – Prescott *et al.*

X. APPENDIX C – RELATED PROCEEDINGS

None

EXHIBIT 1

US 20030078374A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2003/0078374 A1**

Roberts et al. (43) **Pub. Date: Apr. 24, 2003**

(54) **COMPLEMENTARY PEPTIDE LIGANDS GENERATED FROM THE HUMAN GENOME**

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(52) **U.S. Cl.** **530/350**; 536/23.2; 702/19

(57) **ABSTRACT**

In the current invention the application of our novel informatics approach to the databases containing nucleotide and peptide sequences from the human genome generates the sequence of many peptides which form the basis of an innovative and novel approach to developing new therapeutic agents.

This invention claims the use of specific complementary peptides to the proteins encoded in the human genome as reagents and drugs for drug discovery programmes.

Proteom Antisense Protein Comparison Algorithm FIG 1

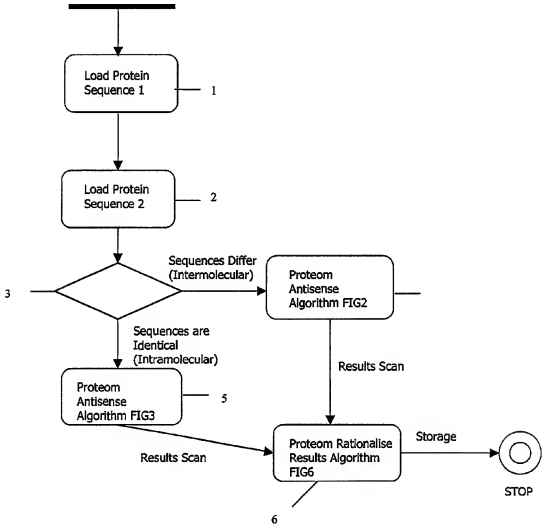


FIG 2

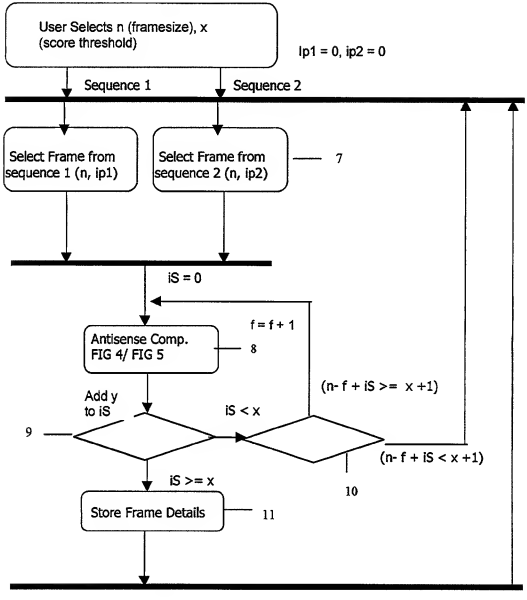
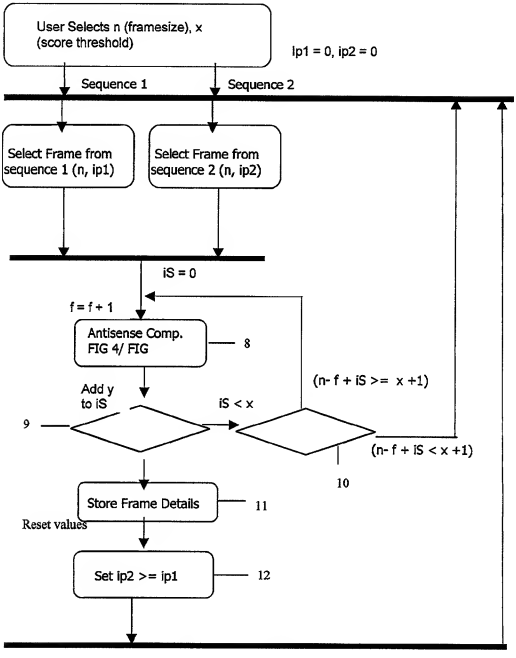
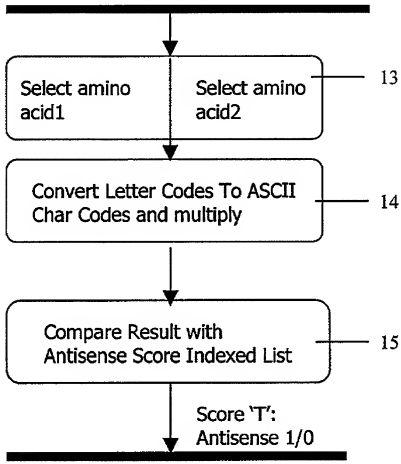

User Selects n (framesize), x (score threshold)
Ip1 = 0, ip2 = 0
Sequence 1
Sequence 2
Select Frame from sequence 1 (n, ip1)
Select Frame from sequence 2 (n, ip2)
7
iS = 0
f = f + 1
Antisense Comp. FIG 4/ FIG 5
8
(n- f + iS >= x +1)
Add y to iS
9
iS < x
(n- f + iS < x +1)
10
iS >= x
Store Frame Details
11

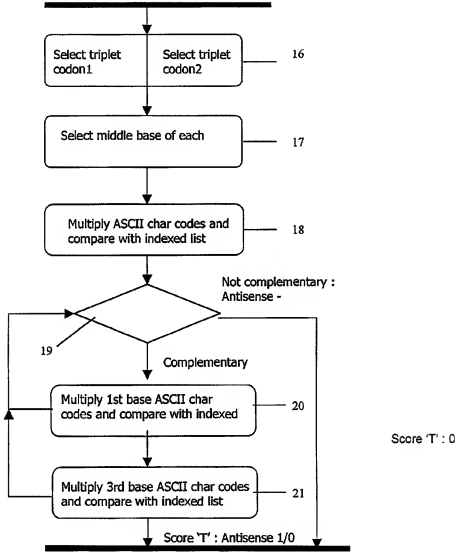
FIG 3


User Selects n (framesize), x (score threshold)
Ip1 = 0, ip2 = 0
Sequence 1
Sequence 2
Select Frame from sequence 1 (n, ip1)
Select Frame from sequence 2 (n, ip2)
iS = 0
f = f + 1
Antisense Comp. FIG 4/ FIG
8
Add y to iS
9
iS < x
(n- f + iS >= x +1)
(n- f + iS < x +1)
10
Store Frame Details
11
Reset values
Set ip2 >= ip1
12

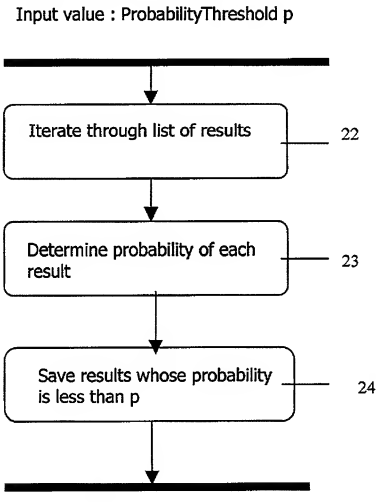
Antisense Matrix Algorithm FIG 4 (Amino Acid Level)



Antisense Matrix Algorithm FIG 5 (DNA Level)



Rationalise Results Algorithm FIG 6 (Amino Acid Level)



COMPLEMENTARY PEPTIDE LIGANDS GENERATED FROM THE HUMAN GENOME

[0001] Specific protein interactions are critical events in most biological processes in health and disease. A clear idea of the way proteins interact, their three dimensional structure and the types of molecules which might block or enhance interaction are critical aspects of the science of drug discovery in the pharmaceutical industry.

[0002] Current predictions estimate that the human genome will be sequenced by 2002 if not sooner. This has accelerated the requirement for informatics tools for mining of the genomic sequence data. A process for the searching and analysis of protein and nucleotide sequence databases has been identified. Significant utility can be acheived within the pharmaceutical i ndustry by searching and analysing protein and nucleotide sequence databases to identify complementary peptides that interact with their relevant target proteins.

[0003] These novel peptides can be used as lead ligands to facilitate drug design and development. This invention describes the application of this process to the databases containing nucleotide and protein sequence data from the human genome.

[0004] This invention claims the use of specific complementary peptides to the proteins encoded in the human genome as reagents and drugs for drug discovery programmes.

BACKGROUND

[0005] Specific protein interactions are critical events in most biological processes and a clear idea of the way proteins interact, their three dimensional structure and the types of molecules which might block or enhance interaction are critical aspects of the science of drug discovery in the pharmaceutical industry.

[0006] Proteins are made up of strings of amino acids and each amino acid in a string is coded for by a triplet of nucleotides present in DNA sequences (Stryer 1997). The linear sequence of DNA code is read and translated by a cell's synthetic machinery to produce a linear sequence of amino acids that then fold to form a complex three-dimensional protein.

[0007] In general it is held that the primary structure of a protein determines its tertiary structure. A large volume of work supports this view and many sources of software are available to the scientists in order to produce models of protein structures (Sansom 1998). In addition, a considerable effort is underway in order to build on this principle and generate a definitive database demonstrating the relationships between primary and tertiary protein structures. This endeavour is likened to the human genome project and is estimated to have a similar cost (Gasterland 1998).

[0008] The binding of large proteinaceous signaling molecules (such as hormones) to cellular receptors regulates a substantial portion of the control of cellular processes and functions. These protein-protein interactions are distinct from the interaction of substrates to enzymes or small molecule ligands to seven-transmembrane receptors. Protein-protein interactions occur over relatively large surface areas, as opposed to the interactions of small molecule ligands with serpentine receptors, or enzymes with their substrates, which usually occur in focused "pockets" or "clefts". Thus, protein-protein targets are non-traditional and the pharmaceutical community has had very limited success in developing drugs that bind to them using currently available approaches to lead discovery. High throughput screening technologies in which large (combinatorial) libraries of synthetic compounds are screened against a target protein(s) have failed to produce a significant number of lead compounds.

[0009] Many major diseases result from the inactivity or hyperactivity of large protein signaling molecules. For example, diabetes mellitus results from the absence or ineffectiveness of insulin, and dwarfism from the lack of growth hormone. Thus, simple replacement therapy with recombinant forms of insulin or growth hormone heralded the begirnings of the biotechnology industry. However, nearly all drugs that target protein-protein interactions or that mimic large protein signaling molecules are also large proteins. Protein drugs are expensive to manufacture, difficult to formulate, and must be given by injection or topical administration.

[0010] It is generally believed that because the binding interfaces between proteins are very large, traditional approaches to drug screening or design have not been successful. In fact, for most protein-protein interactions, only small subsets of the overall intermolecular surfaces are important in defining binding affinity.

[0011] "One strongly suspects that the many crevices, canyons, depressions and gaps, that punctuate any protein surface are places that interact with numerous micro- and macro-molecular ligands inside the cell or in the extracellular spaces, the identity of which is not known" (Goldstein 1998).

[0012] Despite these complexities, recent evidence suggests that protein-protein interfaces are tractable targets for drug design when coupled with suitable functional analysis and more robust molecular diversity methods. For example, the interface between hGH and its receptor buries ~1300 Sq. Angstroms of surface area and involves 30 contact side chains across the interface. However, alanine-scanning mutagenesis shows that only eight side-chains at the center of the interface (covering an area of about 350 Sq. Angstroms) are crucial for affinity. Such "hot spots" have been found in numerous other protein-protein complexes by alanine-scanning, and their existence is likely to be a general phenomenon.

[0013] The problem is therefore to define the small subset of regions that define the binding or functionality of the protein.

[0014] The important commercial reason for this is that a more efficient way of doing this would greatly accelerate the process of drug development.

[0015] These complexities are not insoluble problems and newer theoretical methods should not be ignored in the drug design process. Nonetheless, in the near future there are no good algorithms that allow one to predict protein binding affinities quickly, reliably, and with high precision (Sunesis website www.sunesis.com Sep. 17, 1999).

[0016] A process for the analysis of whole genome databases has been developed. Significant utility can be achieved

within the pharmaceutical industry by searching and analyzing protein and nucleotide sequence databases to identify complementary peptides which interact with their relevant target proteins.

[0017] These novel peptides can be used as lead ligands to facilitate drug design and development. This invention describes the application of this process to databases containing nucleotide and protein sequence data from the human genome.

[0018] The process has been described in patent application number GB 9927485.4, filed Nov. 19, 1999 for use in analysing, and manipulating the sequence data (both DNA and protein) found in large databases and its utility in conducting systematic searches to identify the sequences which code for the key intermolecular surfaces or "hot spots" on specific protein targets.

[0019] This technology will have significant applications in the application of informatics to sequence databases in order to identify lead molecules for numerous important pharmaceutical targets.

THE INVENTION

[0020] In the current invention the application of our novel informatics approach to the databases containing nucleotide and peptide sequences from the human genome generates the sequence of many peptides which form the basis of an innovative and novel approach to developing new therapeutic agents.

[0021] This invention claims the use of specific complementary peptides to the proteins encoded in the human genome as reagents and drugs for drug discovery programmes.

APPLICATION OF THE DATA MINING PROCESS TO THE ANALYSIS OF THE HUMAN GENOME

[0022] One of the key aims of the Human Genome Project is to identify all of the 80,000 to 140,000 genes in human DNA and to determine the complete sequence of the genome (3 billion bases). The first working draft of the human genome sequence (90% coverage) is likely to be completed by 2000 with the finished sequence being completed by 2002. The public availability of this sequence has provided a resource that can now be mined using novel informatics technologies.

[0023] Most human genes are expressed as multiple distinct proteins. It has been estimated that the number of actual proteins generated by the human genome is at least ten times greater. The data mining process described, patent application number GB 9927485.4 greatly accelerates the pace of identification and optimization of small peptides that bind to protein-protein targets. This provides a means of reducing the complexity of the human genetic information by identifying those regions of proteins that are likely to be important targets for drug development. In addition, the computational methods identify proteins that are functionally linked through different pathways or structural complexes.

[0024] We have applied our computational approach with its novel algorithms for generating complementary peptides, patent application number GB 9927485.4, to the human genome. Human nucleotide and protein sequence data is publicly available in a number of large databases (see EXAMPLE 1), and these are continually updated as more sequence becomes available. The identification of novel complementary peptides will allow new lead ligands to enhance drug design and discovery.

[0025] The biological relevance of this approach is described (EXAMPLE 2) and the utility of peptides as tools for functional genomics studies is outlined in EXAMPLE 3.

[0026] A catalogue of complementary inter-molecular peptides frame size 10 (average 3 per gene) was generated for each gene within the human genome (see EXAMPLE 4).

[0027] Sets of shorter 'daughter' sequences of frame size 5, 6, 7, 8 or 9 can also be derived from these sequences (EXAMPLE 5).

[0028] A further set of intra-molecular complementary peptide sequences was also generated for each gene within the human genome (see EXAMPLE 6).

[0029] Sets of shorter 'daughter' sequences of frame size 5, 6, 7, 8 or 9 can also be derived from these sequences (EXAMPLE 7).

[0030] Each complementary peptide sequence has a unique identifying number in the catalog and peptides are categorised as either intra-molecular or inter-molecular peptides within the human genome as shown in the table below (and in EXAMPLES 4 and 6):

Genome	Inter-molecular peptides	Intra-molecular peptides
Human	1-3622	3624-4203

[0031] Utilizing our novel approach we were able to discover the sequences of complementary peptides that have the potential to interact with and alter the functionality of the relevant protein coded for by its gene. Furthermore the second analysis provides information as to the regions on other proteins which might interact with the first protein (its 'molecular partners' in physiological functions).

[0032] The peptide sequences described in this patent can be readily made into peptides by a multitude of methods. The peptides made from the sequences described in this patent will have considerable utility as tools for functional genomics studies, reagents for the configuration of high-throughput screens, a starting point for medicinal chemistry manipulation, peptide mimetics, and therapeutic agents in their own right.

[0033] The process of patent application number GB9927485.4 will now be described below. The examples of this present application are the result of applying that process to a selected human database: it will readily be appreciated that use of the process on other databases will yield peptide sequences and catalogues of intra- and inter-molecular complementary peptides specific to the other human databases (e.g. the databases in EXAMPLE 1).

[0034] The current problems associated with design of complementary peptides are:

[0035] A lack of understanding of the forces of recognition between complementary peptides

[0036] An absence of software tools to facilitate searching and selecting complementary peptide pairs from within a protein database

[0037] A lack of understanding of statistical relevance/distribution of naturally encoded complementary peptides and how this corresponds to functional relevance.

[0038] Based on these shortfalls, our process provides the following technological advances in this field:

[0039] A mini library approach to define forces of recognition between human Interleukin (IL) 1β and its complementary peptides.

[0040] A high throughput computer system to analysc an entire database for intra/inter-molecular complementary regions.

[0041] Studies into preferred complementary peptide pairings between IL-1β and its complementary ligand reveal the importance of both the genetic code and complementary hydropathy for recognition. Specifically, for our example, the genetic code for a region of protein codes for the complementary peptide with the highest affinity. An important observation is that this complementary peptide maps spatially and by residue hydropathic character to the interacting portion of the IL-1R receptor, as elucidated by the X-ray crystal structure Brookhaven reference pdb2itb.ent.

[0042] Using these novel observations as guiding principles for analysis, we have developed a computational analysis system to evaluate the statistical and functional relevance of intra/inter-molecular complementary sequences.

[0043] This process provides significant benefits for those interested in:

[0044] The analysis and acquisition of peptide sequences to be used in the understanding of protein-protein interactions.

[0045] The development of peptides or small molecules which could be used to manipulate these interactions.

[0046] The advantages of this process to previous work in this field include:

[0047] Using a valid statistical model. Previously, complementary mappings within protein structures has been statistically validated by assuming that the occurrence of individual amino acids is equally weighted at 1⁄20 (Baranyi, 1995). Our statistical model takes into account the natural occurrence of amino acids and thus generates probabilities dependent on sequence rather than content per se.

[0048] Facilitation of batch searching of an entire database. Previously, investigations into the significance of naturally encoded complementary related sequences have been limited to small sample sizes with non-automated methods. The invention allows for analysis of an entire database at a time, overcoming the sampling problem, and providing for the first time an overview or 'map' of complementary peptide sequences within known protein sequences.

[0049] The ability to map complementary sequences as a function of frame size and percentage antisense amino acid content. Previously, no consideration has been given to the significance of the frame length of complementary sequences. Our process produces a statistical map as a function of frame size and percentage complementary residue content such that the statistical importance of how nature selects these frames may be evaluated.

BRIEF DESCRIPTION OF DRAWINGS

[0050] The process is described with reference to accompanying drawings. In the drawings, like reference numbers indicate identical or functionally similar elements.

[0051] FIG. (1) shows a block diagram illustrating one embodiment of a method of the present invention

[0052] FIG. (2) shows a block diagram illustrating one embodiment for carrying out Step 4 in FIG. (1)

[0053] FIG. (3) shows a block diagram illustrating one embodiment for carrying out Step 5 in FIG. (1)

[0054] FIG. (4) shows a block diagram illustrating one embodiment for carrying out Step 8 in FIGS. (2) and (3)

[0055] FIG. (5) shows a block diagram illustrating one embodiment for carrying out Step 8 in FIGS. (2) and (3)

[0056] FIG. (6) shows a block diagram illustrating one embodiment for carrying out Step 6 in FIG. (1)

A DESCRIPTION OF THE ANALYTICAL PROCESS

[0057] The software, ALS (antisense ligand searcher), performs the following tasks:

[0058] Given the input of two amino acid sequences, calculates the position, number and probability of the existence of intra- (within a protein) and inter- (between proteins) molecular antisense regions. 'Antisense' refers to relationships between amino acids specified in EXAMPLES 8 and 9 (both 5'->3' derived and 3'->5' derived coding schemes).

[0059] Allows sequences to be inputted manually through a suitable user interface (UI) and also through a connection to a database such that automated, or batch, processing can be facilitated.

[0060] Provides a suitable database to store results and an appropriate interface to allow manipulation of this data.

[0061] Allows generation of random sequences to function as experimental controls.

[0062] Diagrams describing the algorithms involved in this software are shown in FIGS. 1-5.

DETAILED DESCRIPTION

[0063] 1. Overview

[0064] The present process is directed toward a computer-based process, a computer-based system and/or a computer

program product for analysing antisense relationships between protein or DNA sequences. The method of the embodiment provides a tool for the analysis of protein or i DNA sequences for antisense relationships. This embodiment covers analysis of DNA or protein sequences for intramolecular (within the same sequence) antisense relationships or inter-molecular (between 2 different sequences) antisense relationships. This principle applies whether the sequence contains amino acid information (protein) or DNA information, since the former may be derived from the latter.

[0065] The overall process is to facilitate the batch analysis of an entire genome (collection of genes/and or protein sequences) for every possible antisense relationship of both inter- and intra-molecular nature. For the purpose of example it will be described here how a protein sequence database may be analysed by the methods described.

[0066] The program runs in two modes. The first mode (Intermolecular) is to select the first protein sequence in the databases and then analyse the antisense relationships between this sequence and all other protein sequences, one at a time. The program then selects the second sequence and repeats this process. This continues until all of the possible relationships have been analysed. The second mode (Intramolecular) is where each protein sequence is analysed for antisense relationships within the same protein and thus each sequence is loaded from the database and analysed in turn for these properties. Both operational modes use the same core algorithms for their processes. The core algorithms are described in detail below.

[0067] An example of the output from this process is a list of proteins in the database that contain highly improbable numbers of intramolecular antisense frames of size 10 (frame size is a section of the main sequence, it is described in more detail below).

[0068] 2. Method

[0069] For the purpose of example protein sequence 1 is ATRGRDSRDERSDERTD and protein sequence 2 is GTFRTSREDSTYSGDTDFDE (universal 1 letter amino acid codes used).

[0070] In step **1** (see **FIG. 1**), a protein sequence, sequence 1, is loaded. The protein sequence consists of an array of universally recognised amino acid one letter codes, e.g. 'ADTRGSRD'. The source of this sequence can be a database, or any other file type. Step 2, is the same operation as for step **1**, except sequence 2 is loaded. Decision step 3 involves comparing the two sequences and determining whether they are identical, or whether they differ. If they differ, processing continues to step **4**, described in **FIG. 2**, otherwise processing continues to step **5**, described in **FIG. 3**.

[0071] Step **6** analyses the data resulting from either step **4**, or step **5**, and involves an algorithm described in **FIG. 6**.

[0072] Description of parameters used in **FIG. 2**

Name	Description
N	Framesize—the number of amino acids that make up each 'frame'
X	Score threshold—the number of amino acids that have to fulfil the antisense criteria within a given frame for that frame to be stored for analysis

-continued

Name	Description
Y	Score of individual antisense comparison (either 1 or 0)
iS	Running score for frame—(sum of y for frame)
ip1	Position marker for Sequence 1—used to track location of selected frame for sequence 1
ip2	Position marker for Sequence 2—used to track location of selected frame for sequence 1
F	Current position in frame

[0073] In Step **7**, a 'frame' is selected for each of the proteins selected in steps **1** and **2**. A 'frame' is a specific section of a protein sequence. For example, for sequence 1, the first frame of length '5' would correspond to the characters 'ATRGR'. The user of the program decides the frame length as. an input value. This value corresponds to parameter 'n' in **FIG. 2**. A frame is selected from each of the protein sequences (sequence 1 and sequence 2). Each pair of frames that are selected are aligned and frame position parameter f is set to zero. The first pair of amino acids are 'compared' using the algorithm shown in **FIG. 4/FIG. 5**. The score output from this algorithm (y, either one or zero) is added to a aggregate score for the frame iS. In decision step **9** it is determined whether the aggregate score iS is greater than the Score threshold value (x). If it is then the frame is stored for further analysis. If it is not then decision step **10** is implemented. In decision step **10**, it is determined whether it is possible for the frame to yield the score threshold (x). If it can, the frame processing continues and f is incremented such that the next pair of amino acids are compared. If it cannot, the loop exits and the next frame is selected. The position that the frame is selected from the protein sequences is determined by the parameter ip1 for sequence 1 and ip2 for sequence 2 (refer to **FIG. 2**). Each time steps **7** to **10** or **7** to **11** are completed, the value of ip1 is zeroed and then incremented until all frames of sequence 1 have been analysed against the chosen frame of sequence 2. When this is done, ip2 is then incremented and the value of ip1 is incremented until all frames of sequence 1 have been analysed against the chosen frame of sequence 2. This process repeats and terminates when ip2 is equal to the length of sequence 2. Once this process is complete, sequence 1 is reversed programmatically and the same analysis as described above is repeated. The overall effect of repeating steps **7** to **11** using each possible frame from both sequences is to facilitate step **8**, the antisense scoring matrix for each possible combination of linear sequences at a given frame length.

[0074] **FIG. 3** shows a block diagram of the algorithmic process that is carried out in the conditions described in **FIG. 1**. Step **12** is the only difference between the algorithms **FIG. 2** and **FIG. 3**. In step 12, the value of ip2 (the position of the frame in sequence 2) is set to at least the value of ip1 at all times since as sequence 1 and sequence 2 are identical, if ip2 is less than ip1 then the same sequences are being searched twice.

[0075] **FIGS. 4 and 5** describe the process in which a pair of amino acids (**FIG. 4**) or a pair of triplet codons are assessed for an antisense relationship. The antisense relationships are listed in EXAMPLES 8 and 9. In step **13**, the currently selected amino acid from the current frame of

sequence 1 and the currently selected amino acid from the current frame of sequence 2 (determined by parameter 'f' in FIGS. 2/3) are selected. For example, the first amino acid from the first frame of sequence 1 would be 'A' and the first amino acid from the first frame of sequence 2 i would be 'G'. In step **14**, the ASCII character codes for the selected single uppercase characters are determined and multiplied and, in step **15**, the product compared with a list of precalculated scores, which represent the antisense relationships in EXAMPLES 8 and 9. If the amino acids are deemed to fulfil the criteria for an antisense relationship (the product matches a value in the precalculated list) then an output parameter 'T' is set to 1, otherwise the output parameter is set to zero.

[0076] Steps **16-21** relate to the case where the input sequences are DNA/RNA code rather the protein sequence. For example sequence 1 could be AAATTTAGCATG and sequence 2 could be TTTAAAGCATGC. The domain of the current invention includes both of these types of information as input values, since the protein sequence can be decoded from the DNA sequence, in accordance with the genetic code. Steps **16-21** determine antisense relationships for a given triplet codon. In step **16**, the currently selected triplet codon for both sequences is 'read'. For example, for sequence I the first triplet codon of the first frame would be 'AAA', and for sequence 2 this would be 'TTT'. In step **17**, the second character of each of these strings is selected. in step **18**, the ASCII codes are multiplied and compared, in decision step **19**, to a list to find out if the bases selected are 'complementary', in accordance with the rules of the genetic code. If they are, the first bases are compared in step **20**, and subsequently the third bases are compared in step **21**. Step **18** then determines whether the bases are 'complementary' or not. If the comparison yields a 'non-complementary' value at any step the routine terminates and the output score 'T' is set to zero. Otherwise the triplet codons are complementary and the output score **T=1**.

[0077] **FIG. 6** illustrates the process of rationalising the results after the comparison of 2 protein or 2 DNA sequences. In step **22**, the first 'result' is selected. A result consists of information on a pair of frames that were deemed 'antisense' in **FIG. 2** or **3**. This information includes location, length, score (i.e. the sum of scores for a frame) and frame type (forward or reverse, depending on orientation of sequences with respect to one another). In step **23**, the frame size, the score values and the length of the parent sequence are then used to calculate the probability of that frame existing. The statistics, which govern the probability of any frame existing, are described in the next section and refer to equations 1-4. If the probability is less than a user chosen value 'p', then the frame details are 'stored' for inclusion in the final result set (step **24**).

Statistical Basis of Program Operation

[0078] The number of complementary frames in a protein sequence can be predicted from appropriate use of statistical theory.

[0079] The probability of any one residue fitting the criteria for a complementary relationship with any other is defined by the groupings illustrated in EXAMPLE 8. Thus, depending on the residue in question, there are varying probabilities for the selection of a complementary amino acid. This is a result of an uneven distribution of possible partners. For example possible complementary partners for a tryptophan residue include only proline whilst glycine, serine, cysteine and arginine all fulfil the criteria as complementary partners for threonine. The probabilities for these residues aligning with a complementary match are thus 0.05 and 0.2 respectively. The first problem in fitting an accurate equation to describe the expected number of complementary frames within any sequence is integrating these uneven probabilities into the model. One solution is to use an average value of the relative abundance of the different amino acids in natural sequences. This is calculated by equation 1

$$v=\Sigma R^*N \qquad 1$$

[0080] Where v=probability sum, R=fractional abundance of amino acid in *e. coli* proteins, N=number of complementary partners specified by genetic code.

[0081] This value (p) is calculated as 2.98. The average probability (p) of selecting a complementary amino acid is thus 2.98/20=0.149.

[0082] For a single 'frame' of size (n) the probability (C) of pairing a number of complementary amino acids (r) can be described by the binomial distribution (equation 2)

$$C=\frac{n!}{(n-r)!r!}p^r(1-p)^{n-r} \qquad 2$$

[0083] With this information we can predict that the expected number (Ex) of complementary frames in a protein to be:

$$Ex=2(S-n)^2\frac{n!}{(n-r)!r!}p^r(1-p)^{n-r} \qquad 3$$

[0084] Where S=protein length, n=frame size, r=number of complementary residues required for a frame and p=0.149. If r=n, representing that all amino acids in a frame have to fulfil a complementary relationship, the above equation simplifies to:

$$Ex=2(S-n)^2p^n \qquad 4$$

[0085] For a population of randomly assembled amino acid chains of a predetermined length we would expect the number of frames fulfilling the complementary criteria in the search algorithm to vary in accordance with a normal distribution.

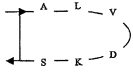
[0086] Importantly, it is possible to standardise results such that given a calculated mean (μ) and standard deviation (σ) for a population it is possible to determine the probability of any specific result occurring. Standardisation of the distribution model is facilitated by the following relation:

$$Z=\frac{X-\mu}{\sigma} \qquad 5$$

[0087] Where X is an single value (result) in a population.

[0088] If we are considering complementary frames with a single protein structure then the above statistical model requires further analysis. In particular, the possibility exists that a region may be complementary to itself, as indicated in the diagram below.

If we are considering complementary frames with a single protein structure then the above statistical model requires further analysis. In particular, the possibility exists that a region may be complementary to itself, as indicated in the diagram below.



Reverse turn motifs within proteins. A region of protein may be complementary to itself. In this scenario, A-S, L-K and V-D are complementary partners. A six amino acid wide frame would thus be reported (in reverse orientation). A frame of this type is only specified by half of the residues in the frame. Such a frame is called a reverse turn.

In this scenario, once half of the frame length has been selected with complementary partners, there is a finite probability that those partners are the sequential neighbouring amino acids to those already selected. The probability of this occurring in any protein of any sequence is:

$$Ex = p^{f/2}(S - f) \qquad 7$$

Where f is the frame size for analysis, and S is the sequence length and p is the average probability of choosing an antisense amino acid.

The software of the embodiment incorporates all of the statistical models reported above such that it may assess whether a frame qualifies as a forward frame, reverse frame, or reverse turn.

[0089] Reverse turn motifs within proteins. A region of protein may be complementary to itself In this scenario, A-S, L-K and V-D are complementary partners. A six amino acid wide frame would thus be reported (in reverse orientation). A frame of this type is only specified by half of the residues in the frame. Such a frame is called a reverse turn.

[0090] In this scenario, once half of the frame length has been selected with complementary partners, there is a finite probability that those partners are the sequential neighbouring amino acids to those already selected. The probability of this occurring in any protein of any sequence is:

$Exp^{f/2}(S-f)$

[0091] Where f is the frame size for analysis, and S is the sequence length and p is the average probability of choosing an antisense amino acid.

[0092] The software of the embodiment incorporates all of the statistical models reported above such that it may assess whether a frame qualifies as a forward frame, reverse frame, or reverse turn.

EXAMPLE 1

Protein and Nucleotide Sequence Databases Amenable for Analysis Using the Process

[0093]

Major Nucleic acid databases

Database	Description	Web site address
Genbank NCBI National Center for Biotechnology Information	The Genbank database is a repository for nucleotide data. The NCBI provides facilities to search for sequences in Genbank by text or by sequence similarity and to submit new sequences.	Http://www.ncbi.nlm.nih.gov/
EMBL	The EMBL database is a repository for nucleotide data. The EBI provides facilities to search for sequences by text or by sequence similarity and to submit new sequences.	http://www.ebi.ac.uk
DbEST	The dbEST database is a repository for Expressed Sequence Tags (EST) data.	http://www.ncbi.nlm.nih.gov/dbEST/
Unigene	The Unigene database is a repository for clustered EST data. UniGene is an experimental system for automatically partitioning EST sequences into a non-redundant set of gene-oriented clusters. Each UniGene cluster contains sequences that represent a unique gene, as well as related information such as the tissue types in which the gene has been expressed and map location. Unigene is split up in sections, catagorized by species origin. The current three sections are Human (hsainigene), Mouse (mmunigene) and Rat (munigene) EST clusters.	http://www.ncbi.nlm.nih.gov/UniGene/
STACK	STACK is a public database of sequences expressed in the human genome. The STACK project aims to make the most comprehensive representation of the sequence of each of the expressed genes in the human genome, by extensive processing of gene fragments to make accurate alignments, highlight errors and provide a carefully joined set of consensus sequences for each gene. A new method to extensively process gene fragments to make accurate alignment, prevent errors and provide a carefully joined set consensus sequences for each gene.	http://www.sanbi.ac.za/Dbases.html

[0094]

Major Protein Sequence databases

Database	Description	URL
SWISS-PROT	Curated protein sequence database which strives to provide a high level of annotations (such as the description of the function of a protein, its domains structure, post-translational	http://www.expasy.ch/sprot/sprot-top.html

-continued

Major Protein Sequence databases

Database	Description	URL
	modifications, variants, etc), a minimal level of redundancy and high level of integration with other databases.	
TrEMBL	Supplement of SWISS-PROT that contains all the translations of EMBL nucleotide sequence entries not yet integrated in SWISS-PROT.	http://www.expasy.ch/sprot/sprot-top.html
OWL	Non-redundant composite of 4 publicly available primary sources: SWISS-PROT, PIR (1-3), GenBank (translation) and NRL-3D. SWISS-PROT is the highest priority source, all others being compared against it to eliminate identical and trivially different sequences. The strict redundancy criteria render OWL relatively "small" and hence efficient in similarity searches.	http://www.biochem.ucl.ac.uk/bsm/dbbrowser/OWL/OWL.html
PIR Protein Information Resource	A comprehensive, annotated, and non-redundant set of protein sequence databases in which entries are classified into family groups and aligments of each group are available.	http://pir.georgetown.edu/
SPTR	Comprehensive protein sequence database that combines the high quality of annotation in SWISS-PROT with the completeness of the weekly updated translation of protein coding sequences from the EMBL nucleotide database.	http://bioinformer.ebi.ac.uk/newsletter/archives/4/sptr.html
NRL_3D	The NRL_3D database is produced by PIR from sequence and annotation information extracted from the Brookhaven Protein Databank (PDB) of crystallographic 3D structures.	http://www-nbrf.georgetown.edu/pirwww/search/textarl3d.html

EXAMPLE 2

Algorithm Determined Sequence In IL-1 Receptor Binding to IL-1β

[0095] The programme identified the antisense region LITVLNI in the interleukin 1 type 1 receptor (IL-1R). The biological relevance of this peptide has been demonstrated and these findings are summarised below:

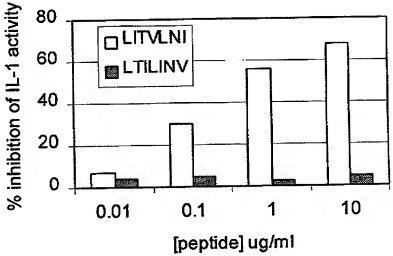
[0096] Program picked out antisense region LITVLNI in the IL-1R receptor.

[0097] This peptide was shown to inhibit the biological activity of IL-1β in two independent in vitro bioassays.

[0098] The effect is dependent on the peptide sequence.

[0099] The same effect is also seen in a Serum Amyloid IL-1 assay (i.e. assay independence).

[0100] The peptide was shown to bind directly to IL-1 by using biosensing techniques



EXAMPLE 3

Demonstration of the Utility of the Process when Applied to the Human Genome

[0101] 1. DNA-Binding Proteins

[0102] Sequence-specific DNA binding by proteins controls transcription (Pabo and Sauer, 1992), recombination (Craig, 1988), restriction (Pingoud and Jeltsch, 1997) and replication (Margulies and Kaguni, 1996). Sequence requirements are usually determined by assays that measure the effects of mutations on binding of DNA and amino acid residues implicated in these interactions.

[0103] The central role of DNA binding proteins in the cell cycle means they have a key role in cell proliferation, tumour formation and progression.

[0104] The identification of anti-sense peptides targetted to such proteins have the potential to be useful targets for the development of therapeutic compounds for the treatment of cancer.

[0105] For instance, Koivunen et al., 1999, identified a novel cyclic decapeptide that not only targetted angiogenic (developing) blood vessels but also inhibited the matrix metalloproteinases MMP-2 and MMP-9 (MMP activity is a requirement of tumour growth, angiogenesis and metastasis). The specificity of this novel peptide for MMP-2 and MMP-9 but not other metalloproteinases suggested it might prove useful in tumour therapy. When injected into mice the peptide impeded both growth and invasion of established tumours.

[0106] This research demonstrates the potential for using specific peptides as agents for targetting tumours and as anticancer therapies.

[0107] 2. The Human Major Histocompatibility Complex

[0108] The human major histocompatibility complex is associated with more diseases than any other region of the human genome, including most autoimmune conditions (e.g. diabetes and rheumatoid arthritis). A search of OMIM retrieved 187 entries under Major Histocompatibility Complex, associated with phenotypes such as multiple sclerosis, coeliac disease, Graves disease and alopecia.

[0109] The first complete sequence of the human MHC region on chromosome 6 has recently been determined (The MHC sequencing consortium, 1999). Over 200 gene loci were identified making this the most gene-dense region of the human genome sequenced so far. Of these, many are of unknown function but at least 40% of the 128 genes predicted to be expressed are involved in immune system function. It also encodes the most polymorphic proteins, the class I and class II molecules, some of which have over 200 allelic variants. This extreme polymorphism is thought to be driven and maintained by the conflict between the immune system and infectious pathogens.

[0110] The importance of this region to human disease makes it an ideal target for analysis to identify novel therapeutic peptides.

EXAMPLE 4

[0111] The human genome, which is estimated to contain between 80,000 and 140,000 genes was screened for intermolecular peptides using the method described in patent application number GB 9927485.4, filed Nov. 19, 1999. The gene, database accession number, its predicted interacting peptides and their position within the coding sequence of the gene are shown in the attached sequence listing: SEQ ID Nos. [1-3622].

EXAMPLE 5

Derivation of Daughter Sequences from Parent Sequences

[0112] For each pair of 'frames' of amino acids which are deemed a 'hit' by the algorithm the current invention includes derived pairs of composite daughter sequences of shorter frame lengths which automatically fulfil the same 'complementary' relationship.

[0113] For example, there is a complementary frame of size 10 between genes (inter-molecular) CBFA2 and ACTR3 of Homo Sapien.:

GENE1	GENE2	Sequence 1	Location	Sequence 2	Location	Score
CBFA2	ACTR3	DLRFVGRSGR	133–142	PTAAPDKTEV	77–86	10

[0114] One embodiment of the invention covers the derivation of the following sequences at frame length of 5:

GENE	GENE2	Sequence 1	Location	Sequence 2	Location	Score
CBFA2	ACTR3	DLRFV	133–137	VETKD	77–81	5
CBFA2	ACTR3	LRFVG	134–138	ETKDP	78–82	5
CBFA2	ACTR3	RFVGR	135–139	TKDPA	79–83	5
CBFA2	ACTR3	FVGRS	136–140	KDPAA	80–84	5
CBFA2	ACTR3	VGRSG	137–141	DPAAT	81–85	5
CBFA2	ACTR3	GRSGR	138–142	PAATP	82–86	5

[0115] One embodiment of the invention covers the derivation of the following sequences at frame length of 6:

GENE	GENE2	Sequence 1	Location	Sequence 2	Location	Score
CBFA2	ACTR3	DLRFVG	133–138	VETKDP	77–82	6
CBFA2	ACTR3	LRFVGR	134–139	ETKDPA	78–83	6
CBFA2	ACTR3	RFVGRS	135–140	TKDPAA	79–84	6
CBFA2	ACTR3	FVGRSG	136–141	KDPAAT	80–85	6
CBFA2	ACTR3	VGRSGR	137–142	DPAATP	81–86	6

[0116] One embodiment of the invention covers the derivation of the following sequences at frame length of 7:

GENE	GENE2	Sequence 1	Location	Sequence 2	Location	Score
CBFA2	ACTR3	DLRFVGR	133–139	VETKDPA	77–83	7
CBFA2	ACTR3	LRFVGRS	134–140	ETKDPAA	78–84	7
CBFA2	ACTR3	RFVGRSG	135–141	TKDPAAT	79–85	7
CBFA2	ACTR3	FVGRSGR	136–142	KDPAATP	80–86	7

[0117] One embodiment of the invention covers the derivation of the following sequences at frame length of 8:

GENE	GENE2	Sequence 1	Location	Sequence 2	Location	Score
CBFA2	ACTR3	DLRFVGRS	133–140	VETKDPAA	77–84	8
CBFA2	ACTR3	LRFVGRSG	134–141	ETKDPAAT	78–85	8
CBFA2	ACTR3	RFVGRSGR	135–142	TKDPAATP	79–86	8

[0118] One embodiment of the invention covers the derivation of the following sequences at frame length of 9:

GENE	GENE2	Sequence 1	Location	Sequence 2	Location	Score
CBFA2	ACTR3	DLRFVGRSG	133–141	VETKDPAAT	77–85	9
CBFA2	ACTR3	LRFVGRSGR	134–142	ETKDPAATP	78–86	9

EXAMPLE 6

[0119] The human genome, which is estimated to contain between 80,000 and 140,000 genes was screened for intramolecular peptides using the method described in patent application number GB 9927485.4, filed Nov. 19, 1999. The gene, database accession number, its predicted interacting peptides and their position within the coding sequence of the gene are shown in the attached sequence listing: SEQ ID Nos. [3624-4203].

EXAMPLE 7

Derivation of Daughter Sequences from Parent Sequences

[0120] For each pair of 'frames' of amino acids which are deemed a 'hit' by the algorithm the current invention includes derived pairs of composite daughter sequences of shorter frame lengths which automatically fulfil the same 'complementary' relationship.

[0121] For example, gene ADRAIB in Homo Sapiens contains the following intra-molecular complementary relationship of frame length 10:

GENE	Sequence 1	Location	Sequence 2	Location	Score
ADRA1B	GGGSAGGAAP	28–37	GGGSAGGAAP	28–37	10

[0122] One embodiment of the invention covers the derivation of the following sequences at frame length of 5:

GENE	Sequence 1	Location	Sequence 2	Location	Score
ADRA1B	GGGSA	28–32	PAAGG	37–33	5
ADRA1B	GGSAG	29–33	AAGGA	36–32	5
ADRA1B	GSAGG	30–34	AGGAS	35–31	5
ADRA1B	SAGGA	31–35	GGASG	34–30	5
ADRA1B	AGGAA	32–36	GASGG	33–29	5
ADRA1B	GGAAP	33–37	ASGGG	32–28	5

[0123] One embodiment of the invention covers the derivation of the following sequences at frame length of 6:

GENE	Sequence 1	Location	Sequence 2	Location	Score
ADRA1B	GGGSAG	28–33	PAAGGA	37–32	6
ADRA1B	GGSAGG	29–34	AAGGAS	36–31	6
ADRA1B	GSAGGA	30–35	AGGASG	35–30	6
ADRA1B	SAGGAA	31–36	GGASGG	34–29	6
ADRA1B	AGGAAP	32–37	GASGGG	33–28	6

[0124] One embodiment of the invention covers the derivation of the following sequences at frame length of 7:

GENE	Sequence 1	Location	Sequence 2	Location	Score
ADRA1B	GGGSAGG	28–34	PAAGGAS	37–31	7
ADRA1B	GGSAGGA	29–35	AAGGASG	36–30	7
ADRA1B	GSAGGAA	30–36	AGGASGG	35–29	7
ADRA1B	SAGGAAP	31–37	GGASGGG	34–28	7

[0125] One embodiment of the invention covers the derivation of the following sequences at frame length of 8:

GENE	Sequence 1	Location	Sequence 2	Location	Score
ADRA1B	GGGSAGGA	28–35	PAAGGASG	37–30	8
ADRA1B	GGSAGGAA	29–36	AAGGASGG	36–29	8
ADRA1B	GSAGGAAP	30–37	AGGASGGG	35–28	8

[0126] One embodiment of the invention covers the derivation of the following sequences at frame length of 9:

GENE	Sequence 1	Location	Sequence 2	Location	Score
ADRA1B	GGGSAGGAA	28–36	PAAGGASGG	37–29	9
ADRA1B	GGSAGGAAP	29–37	AAGGASGGG	36–28	9

EXAMPLE 8

The Amino Acid Pairings Resulting from Reading the Anticodon for Naturally Occuring Amino Acid Residues in the 5'-3' Direction

[0127]

Amino Acid	co-don	Complementary codon	Complementary Amino acid	Amino Acid	co-don	Complementary codon	Complementary Amino acid
Alanine	GCA	UGC	Cysteine	Serine	UCA	UGA	Stop
	GCG	CGC	Arginine		UCC	GGA	Glycine
	GCC	GGC	Glycine		UCG	CGA	Arginine

-continued

Amino Acid	co-don	Complementary codon	Complementary Amino acid	Amino Acid	co-don	Complementary codon	Complementary Amino acid
	GCU	AGC	Serine		UCU	AGA	Arginine
					AGC	GCU	Alanine
					AGU	ACU	Threonine
Arginine	CGG	CCG	Proline	Glutamine	CAA	UUG	Leucine
	CGA	UCG	Serine		CAG	CUG	Leucine
	CGC	GCG	Alanine				
	CGU	ACG	Threonine				
	AGG	CCU	Proline				
	AGA	UCU	Serine				
Aspartic Acid	GAC	GUC	Valine	Glycine	GGA	UCC	Serine
	GAU	AUC	Isoleucine		GGC	GCC	Alanine
					GGU	ACC	Threonine
					GGG	CCC	Proline
Asparagine	AAC	GUU	Valine	Histidine	CAC	GUG	Valine
	AAU	AUU	Isoleucine		CAU	AUG	Methionine
Cysteine	UGU	ACA	Threonine	Isoleucine	AUA	UAU	Tyrosine
	UGC	GCA	Alanine		AUC	GAU	Aspartic acid
					AUU	AAU	Asparagine
Glutamic Acid	GAA	UUC	Phenylalanine	Leucine	CUG	CAG	Glutamine
	GAG	CUC	Leucine		CUC	GAG	Glutamic acid
					CUU	AAG	Lysine
					UUA	UAA	Stop
					CUA	UAG	Stop
					UUG	CAA	Glutamine
					CUG	CAG	Glutamine
Lysine	AAA	UUU	Phenylalanine	Threonine	ACA	UGU	Cysteine
	AAG	CUU	Leucine		ACG	CGU	Arginine
					ACC	GGU	Glycine
					ACU	AGU	Serine
Methionine	AUG	CAU	Histidine	Tryptophan	UGG	CCA	Proline
Phenylalanine	UUU	AAA	Lysine	Tyrosine	UAC	GUA	Valine
	UUC	GAA	Glutamic Acid		UAU	AUA	Isoleucine
Proline	CCA	UGG	Tryptophan	Valine	GUA	UAC	Tyrosine
	CCC	GGG	Glycine		GUG	CAC	Histidine
	CCU	AGG	Arginine		GUC	GAC	Aspartic Acid
	CCG	CGG	Arginine		GUU	AAC	Asparagine

EXAMPLE 9

[0128] The relationships between amino acids and the residues encoded in the complementary strand reading 3'-5'

Amino Acid	co-don	Complementary codon	Complementary Amino acid	Amino Acid	co-don	Complementary codon	Complementary Amino acid
Alanine	GCA	CGU	Arginine	Serine	UCA	AGU	Serine
	GCG	CGC			UCC	AGG	Arginine
	GCC	CGG			UCG	AGC	Serine
	GCU	CGA			UCU	AGA	Arginine
					AGC	UCG	Serine
					AGU	UCA	Serine
Arginine	CGG	GCC	Alanine	Glutamine	CAA	GUU	Valine
	CGA	GCU	Alanine		CAG	GUC	Valine

-continued

Amino Acid	co-don	Comple-mentary codon	Complementary Amino acid	Amino Acid	co-don	Comple-mentary codon	Comple-mentary Amino acid
	CGC	GCG	Alanine				
	CGU	GCA	Alanine				
	AGG	UCC	Serine				
	AGA	UCU	Serine				
Aspartic Acid	GAC	GUC	Valine	Glycine	GGA	CCU	Proline
	GAU	AUC	Isoleucine		GGC	CCG	Proline
					GGU	CCA	Proline
					GGG	CCC	Proline
Asparagine	AAC	UUG	Leucine	Histidine	CAC	GUG	Valine
	AAU	UUA	Leucine		CAU	GUA	Valine
Cysteine	UGU	ACA	Threonine	Isoleucine	AUA	UAU	Tyrosine
	UGC	ACG	Threonine		AUC	UAG	Stop
					AUU	UAA	Stop
Glutamic	GAA	CUU	Leucine	Leucine	CUG	GAC	Asp
Acid	GAG	CUG	Leucine		CUC	GAG	Glutamic
					CUU	GAA	acid
					UUA	AAU	Glutamic
					CUA	GAU	Acid
					UUG	AAC	Asparagine
					CUG	GAC	Aspartic
							Acid
							Asparagine
							Aspartic
							Acid
Lysine	AAA	UUU	Phenylalanine	Threonine	ACA	UGU	Cysteine
	AAG	UUC	Phenylalanine		ACG	UGC	Cysteine
					ACC	UGG	Tryptophan
					ACU	UGA	Stop
Methionine	AUG	UAC	Tyrosine	Tryptophan	UGG	ACC	Threonine
Phenylalanine	UUU	AAA	Lysine	Tyrosine	UAC	AUG	Methionine
	UUC	AAG	Lysine		UAU	AUA	Isoleucine
Proline	CCA	GGU	Glycine	Valine	GUA	CAU	Histidine
	CCC	GGG	Glycine		GUG	CAC	Histidine
	CCU	GGA	Glycine		GUC	CAG	Glutamine
	CCG	GGC	Glycine		GUU	CAA	Glutamine

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[0129] All publications, patents, and patent applications cited are hereby incorporated by reference in their entirety.

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SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/sequence.html?DocID=20030078374). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

We claim:

1. A set of peptide ligands; said set consisting of specific complementary peptides to proteins encoded by genes of the human genome.

2. A set of peptide ligands according to claim **1**, wherein the sequences of the peptides in the set are intra-molecular complementary peptide sequences.

3. A set of peptide ligands according to claim **1**, wherein the sequences of the peptides in the set are inter-molecular complementary peptide sequences.

4. A novel peptide having a sequence which is a member of a set according to any preceding claim, capable of antagonising or agonising a specific interaction of a protein with another protein or receptor.

5. Use of a set of peptides according to any of claims 1 to 3 in an assay for screening and identification of one or more peptides according to claim 4.

6. Use according to claim 5 wherein the identified peptide(s) is a drug candidate.

7. Use according to claim 5 wherein the identified peptide(s) is a pro-drug.

8. A partly or wholly non-peptide mimetic of a peptide drug candidate or pro-drug according to claim 4, **6** or **7**, identified by use of the set of peptides according to claim 5.

9. A method for processing sequence data comprising the steps of;

selecting a first protein sequence and a second protein sequence;

selecting a frame size corresponding to a number of sequence elements

such as amino acids or triplet codons, a score threshold, and a frame existence probability threshold;

comparing each frame of the first sequence with each frame of the second sequence by comparing pairs of sequence elements at corresponding positions within each such pair of frames to evaluate a complementary relationship score for each pair of frames;

storing details of any pairs of frames for which the score equals or exceeds the score threshold;

evaluating for each stored pair of frames the probability of the existence of that complementary pair of frames existing, on the basis of the number of possible complementary sequence elements existing for each sequence element in the pair of frames; and

discarding any stored pairs of frames for which the evaluated probability is greater than the probability threshold; wherein each frame is a peptide sequence of defined length.

10. A method according to claim 9, in which the first sequence is identical to the second sequence and a frame at a given position in the first sequence is only compared with frames in the second sequence at the same given position or at later positions in the second sequence, in order to eliminate repetition of comparisons.

11. A method according to claim 9 or **10**, in which the sequence elements at corresponding positions within each of a pair of frames are compared sequentially, each such pair of sequence elements generating a score which is added to an aggregate score for the pair of frames.

12. A method according to claim 11, in which if the aggregate score reaches the score threshold before all the pairs of sequence elements in the pair of frames have been compared, details of the pair of frames are immediately stored and a new pair of frames is selected for comparison.

13. A method according to any preceding claim, in which the sequence elements are amino acids and pairs of amino acids are compared by using an antisense score list.

14. A method according to any of claims 9 to 12, in which the sequence elements are triplet codons and pairs of codons in corresponding positions within each of the pairs of triplet codons are compared by using an antisense score list.

15. A method for processing sequence data substantially as described herein with reference to FIGS. **1** to **6**.

16. A pair of frames or a list of pairs of frames being the product of the method of any of claims 9 to 15, optionally carried on a computer-readable medium.

17. A frame being the product of the method of any of claims 9 to **15**, optionally carried on a computer-readable medium.

18. A peptide, pair of complementary peptides, or set of peptides, being the peptide(s) having the sequence of the frame(s) of claims **16** or **17**.

19. A method for identifying a peptide drug candidate or pro-drug, which method includes the steps of (i) identifying a set of specific complementary peptides according to any of claims 1 to 4; (ii) screening the set for specific protein interaction activity; and (iii) identifying one or more peptide(s) according to claim **5**.

* * * * *

EXHIBIT 2

CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

I hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

May 5, 2008
Date

Steven L. Highlander

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Lois WEISMAN

Serial No.: 10/791,219

Filed: March 2, 2004

For: INTRACELLULAR SIGNALING PATHWAYS IN DIABETIC SUBJECTS

Group Art Unit: 1653

Examiner: Samuel Liu

Atty. Dkt. No.: IOWA:048US/SLH

Confirmation No.: 3887

SECOND AMENDMENT UNDER 37 C.F.R. §1.116

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

This paper is submitted in response to the Advisory Action mailed on January 7, 2008. A petition for extension of time (three months) and the payment of fees are being submitted with the Appeal Brief. No other fees are believed due in connection with this amendment; however, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to this document, the Commissioner is authorized to deduct said fees from Fulbright & Jaworski L.L.P. Account No.: 50-1212/IOWA:048US/SLH.

Amendments to the Claims begin on page 2 of this response; **Remarks** being on page 5.

AMENDMENTS TO THE CLAIMS

Listing of Claims

The following listing of claims replaces all previous listings or versions thereof:

1-17. (Canceled)

18. (Previously presented) An isolated and purified polypeptide comprising the sequence of SEQ ID NO:3.

19. (Previously presented) The isolated and purified polypeptide of claim 18, wherein said polypeptide is a fusion protein further comprising additional non-Vac14 sequences.

20-23. (Canceled)

24. (Currently amended) An oligopeptide of between **[[**10**]]**15 and ~~about~~ 30 residues, said oligopeptide comprising at least **[[**10**]]**15 consecutive residues of SEQ ID NO:3.

25. (Currently amended) The oligopeptide of claim 24, wherein said oligopeptide is **[[**10, **]]**15, 20, 25, or 30 residues in length.

26. (Currently amended) The oligopeptide of claim 24, wherein the number of consecutive residues is **[[**10, **]]**15, 20, 25, or 30.

27-32. (Canceled)

33. (Withdrawn) A method of identifying a subject at risk of developing diabetes comprising assessing the structure, function or expression of Fab1, Vac14 and/or Fig4 in cells of said subject.

34. (Withdrawn) The method of claim 33, wherein assessing comprises assessing expression.

35. (Withdrawn) The method of claim 34, wherein assessing expression comprises Northern blotting.

36. (Withdrawn) The method of claim 34, wherein assessing expression comprises quantitative RT-PCR.

37. (Withdrawn) The method of claim 34, wherein assessing expression comprises Western blotting.

38. (Withdrawn) The method of claim 34, wherein assessing expression comprises quantitative immunohistochemistry.

39. (Withdrawn) The method of claim 33, wherein assessing comprises assessing activity.

40. (Withdrawn) The method of claim 39, wherein assessing activity comprises measuring $PI(3,5)P_2$.

41. (Withdrawn) The method of claim 40, wherein assessing activity comprises measuring $PI(3,5)P_2$ turnover.

42. (Withdrawn) The method of claim 40, wherein assessing activity comprises measuring $PI(3,5)P_2$ steady state levels.

43. (Withdrawn) The method of claim 40, wherein assessing activity comprises measuring $PI(3,5)P_2$ synthesis.

44. (Withdrawn) The method of claim 40, wherein assessing activity comprises measuring PI(3)P.

45. (Withdrawn) The method of claim 39, wherein assessing activity comprises measuring protein kinase activity.

46. (Withdrawn) The method of claim 33, wherein assessing comprises assessing structure.

47. (Withdrawn) The method of claim 46, wherein assessing structure comprises nucleic acid sequencing.

48. (Withdrawn) The method of claim 47, wherein sequence comprises PCR.

49. (Withdrawn) The method of claim 47, wherein sequence comprises RT-PCR.

50. (Withdrawn) The method of claim 469, wherein assessing structure comprises measuring antibody binding.

51. (Withdrawn) The method of claim 50, wherein measuring antibody binding comprises, RIA, ELISA, Western blot or immunohistochemistry.

52. (Withdrawn) The method of claim 46, wherein assessing structure comprises high stringency nucleic acid hybridization.

53. (Withdrawn) The method of claim 33, further comprising obtaining a cell from said subject.

54. (Withdrawn) The method of claim 53, wherein said cell is a kidney cell, a liver cell, a leukocyte, an adipocyte, or a muscle cell.

55. (Withdrawn) The method of claim 53, further comprising subjecting said cell to stress prior to assessing expression or activity.

56. (Withdrawn) The method of claim 55, wherein stress is osmotic stress.

57. (Withdrawn) The method of claim 55, further comprising subjecting said cell to hormonal stimulation prior to assessing expression or activity.

58. (Withdrawn) The method of claim 57, wherein said hormonal stimulation is insulin stimulation.

59-60. (Canceled)

REMARKS

Claims 18, 19, 24-26, and 33-60 are pending in the application.[1] Applicants renew their request for entry of the attached amendments, which are slightly different than those submitted in the response to final Office Action mailed on December 3, 2007. The amendments are believed to dispose of rejections under 35 U.S.C. §112, second paragraph and 35 U.S.C. §102. Moreover, no new matter nor new issues are raised by the amendments. Therefore, entry of the amendments is believed proper.

The examiner is invited to contact the undersigned attorney at 512-536-3184 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

Steven L. Highlander
Reg. No. 37,642
Attorney for Applicant

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(512) 536-3184

Date: May 5, 2008

[1] The examiner indicated that claim 59 and 60 had been canceled. However, non-entry of the amendment means that these claims are still pending – an amendment cannot be denied in part and entered in part.

Acknowledgement Receipt

The USPTO has received your submission at **12:40:21** Eastern Time on **05-MAY-2008** .

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eFiled Application Information

EFS ID	3251954
Application Number	10791219
Confirmation Number	3887
Title	Intracellular signaling pathways in diabetic subjects
First Named Inventor	Lois Weisman
Customer Number or Correspondence Address	Steven L. Highlander Fulbright & Jaworski L.L.P. Suite 2400 600 Congress Avenue AUSTIN TX 78701 US 5125363184
Filed By	Steven Lee Highlander/Christopher Jackson
Attorney Docket Number	IOWA:048US
Filing Date	02-MAR-2004
Receipt Date	05-MAY-2008
Application Type	Utility under 35 USC 111 (a)

Application Details

Submitted Files	Page Count	Document Description	File Size	Warnings
IOWA048US_AMEND.pdf	5		152796 bytes	PASS

Document Description	Page Start	Page End
Amendment After Final	1	1
Claims	2	4
Applicant Arguments/Remarks Made in an Amendment	5	5

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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EXHIBIT 3

An Update of Immunotherapy for Specific Allergies

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Abstract: Allergic diseases are common, disabling and potentially life threatening. The processes that lead to production of excessive allergen-specific IgE production are highly complex and heterogeneous. While current treatment strategies are limited, recent technological advances have provided a better understanding of underlying disease processes and offered new potential therapeutic targets. Optimal treatment strategies permanently modify underlying inflammatory allergic immune responses (immunotherapy) with long term alleviation of symptoms and minimal side -effects. Although these processes are still not completely understood, methods of modifying allergen recognition by the immune system have already been successful. Here, we review recent developments and future directions in allergen immunotherapy and adjunctive therapies. Specifically, we address the molecular mechanisms of allergen immunotherapy and new techniques including allergen modification, allergen gene vaccination, CpG immunostimulation, and peptide immunotherapy. Other non-allergen specific molecular targets (including receptor, cytokine and IgE targets) which may complement specific immunotherapy are also discussed. Ideally these methods will eventually be replaced by strategies targeting the prevention of allergic responses (immunoprophylaxis).

1. OVERVIEW OF UNDERLYING IMMUNOLOGICAL PROCESSES AND POTENTIAL THERAPEUTIC TARGETS FOR IMMUNOTHERAPY

Many allergens have been found to have enzymatic and other biological activity [1] yet these antigens elicit a tolerance response in the majority of people. Unfortunately, for an increasing number of individuals allergens trigger a type I hypersensitivity characterised by a strong humoral response dominated by IgE. Irrespective of the atopic status of the individual, antigen is taken up by antigen presenting cells (APCs; namely dendritic cells, monocytes/macrophages and B cells) and processed for presentation by MHC Class II molecules to CD4+ T cells. In an atopic individual, the resulting allergen-specific T cells have a Th2 skewed phenotype with the cytokine profile dominated by IL4, IL-5 and IL-13. In contrast, the response from the non-atopic person is characteristically Th1 skewed, being dominated by the prototypic Th1 cytokine IFNγ. Numerous factors favour the development of Th2 type antigen-specific responses including the amount and affinity of antigen and the HLA genotype of the responder, the type of APC and the prevailing cytokine milieu.

Th2 type cytokines support the allergic response in many ways. IL-4 and IL-13 (in conjunction with other cognate interactions between T and B lymphocytes) induce ε class switching and production of IgE by B cells. Allergen can then cross link specific IgE bound to high affinity IgE receptors (FcεRI) on mast cells and basophils inducing the release of vasoactive and inflammatory mediators, including histamine and cytokines. It is these molecules that mediate the early reaction characterised by vascular leakage and inflammation. IgE can also facilitate the uptake of small quantities of allergen by B cells via IgE-mediated antigen focusing utilising the low affinity IgE receptor (CD23) [2]. More recently, dendritic cell populations, such as Langerhans cells have been described to use the high affinity receptor for this process [3]. CD23-mediated IgE-facilitated antigen focusing by B cell is postulated to activate T cells and induce to release of proinflammatory cytokines that mediate the late reaction. The other classical Th2 cytokine, IL-5, supports that maturation and survival of eosinophils that contribute to the inflammatory environment within the tissue.

Thus there are numerous targets for alleviating or abrogating allergic inflammatory responses. Widely used pharmacological interventions generally target the biological mediators released by basophils, mast cells and eosinophils, hence the use of anti-histamine therapy and, more recently, of anti-leukotriene therapy. These treatments are not curative whereas immunotherapy directed to any of the numerous targets identified in the brief description above (and reviewed by [4]) offer the best hope of achieving cure, or at least long term alleviation of clinical symptoms.

Study of the immunological response to immunotherapy has provided some information about the immunological mechanisms underlying successful immunotherapy. It is only as the use of immunotherapy becomes more widespread (more allergens targeted/different routes of administration) that we will be able to fully understand the pivotal

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1568-010X/02 $35.00+.00

mechanisms and how regulation of these may be critical for successful treatment of different allergies.

2. CURRENT USE OF ALLERGEN VACCINATION IN ESTABLISHED ALLERGIC DISEASE

The therapeutic benefits of administering increasing quantities of allergen in sensitised patients was established early last century [5]. Using many varied adaptations of this technique, "vaccination" with allergen [6] has been subsequently used to desensitise patients with allergic rhinitis / conjunctivitis, asthma or insect venom allergy. Until very recently there was no clear understanding of the underlying mechanisms of action, or use of standardised procedures and extracts [6]. Vaccination strategies remain the only established forms of therapy which modify disease progress by altering underlying immune responses.

2.1 Mechanisms of Action (of Subcutaneous Immunotherapy)

There are considerable variations in protocols for allergen vaccination, and the mechanisms of action are likely to vary with differences in allergens, adjuvants, doses, duration and routes of administration. Additional host factors, including the pattern of disease expression are also likely to affect the clinical and immunological response. Allergens have been variously administered through oral, sublingual, or subcutaneous routes. There is very limited information about the comparative efficacy of these different routes. Most of the studies evaluating mechanistic pathways in humans relate to subcutaneous allergen vaccination.

Local tissue effects are also seen early in immunotherapy, and are likely to be the result of mechanisms different from those responsible for the long lived changes in cellular responses. There is early reduction in the numbers and activity of local inflammatory effector cells, including mast cells [7, 8] eosinophils [9, 10] and their inflammatory mediators [11] following nasal allergen challenge. Similar findings are documented in the lower respiratory tract [12].

It is now widely held that the immunological changes which lead to sustained clinical improvement are mediated by changes in T cell function. With the recognition of functional dichotomy in T lymphocyte subsets, it was hypothesised [13] that immunotherapy resulted in relative shift towards Th1 responses (with reciprocal inhibition of allergic Th2 responses). There is now evidence to support this. A number of studies have now shown a decrease in the production of Th2 cytokines (IL-4 and IL-5) and an increase in Th1 (IFNγ) responses with immunotherapy [14-16].

In the initial phases of immunotherapy high dose allergen leads to allergen-specific T cell anergy. This transient anergic state has been seen following allergen vaccination with bee venom, wasp, rag weed, cat, grass pollens (reviewed in [17]). Inhibition of lymphoproliferation and both Th1 and Th2 cytokine responses is apparent within 60 days of commensing treatment [17, 18]. This is an active process, associated with increased CD25 expression and increased IL-10 and IL-12 signalling from tissue macrophages and other antigen presenting cells [19]. IL-10 is believed to play an important role in inducing and maintaining T cell anergy (reviewed in [17]). IL-12 [19] has further inhibitory effects on Th2 cytokine production. Although vaccine induced T cell anergy is transient, when allergen responsive T cell are reactivated their subsequent pattern of cytokine response is crucial and determines if allergen vaccination is successful (Th1) or not (Th2).

Inhibition of T cell function does not immediately prevent the production of IgE which may increase transiently. However, within weeks total and specific IgE levels fall in favour of IgG4 production. Reported alterations in allergen-specific IgE with allergen vaccination are varied and do not correlate well with the therapeutic effects [20-22].

Increased levels of allergen-specific IgG1 and IgG4 subclasses [23] were initially proposed to mediate the effects of immunotherapy by "blocking" IgE [24-26]. There is more recent evidence that IgG antibodies may actually inhibit IgE mediated T cell activation [18]. In untreated allergic individuals allergen-specific IgE facilitates CD23 mediated antigen presentation to CD4+ T cells, promoting activation at extremely low allergen concentrations. Following allergen vaccination, allergen-specific IgG inhibits this process so that a significantly higher concentration of allergen is required for T cell stimulation.

Physiochemical properties of immune interactions are a fundamental determinant of T cell activation. It appears that the strength of the association between the MHC class II, allergen peptide and the T cell receptor dictates the pattern of resulting cytokine responses. Factors which favour strong signalling (high allergen dose / high affinity / high density of MHC) promote Th1 responses (reviewed in [17]). This may in part explain the induction of Th1 responses by high allergen doses as well as the HLA associations, and observed heredity of bee venom allergy.

2.2 Sublingual Immunotherapy

There are ongoing efforts to determine the efficacy of other less invasive delivery routes for vaccination, including oral, intranasal and sublingual vaccination. This is probably of greatest relevance to the growing paediatric population with allergic diseases. While a number of double-blind randomised control trials have demonstrated the safety and clinical benefits of sublingual immunotherapy [27, 28, 29 80, 30], other studies have shown no clinical improvement [31] or effects that were only seen in a subgroups of patients [32]. Relatively few of these studies have involved children [28, 33, 34]. The immunological effects of sublingual allergen vaccination are still not well documented, even though this treatment is in therapeutic use in many centres. Even studies demonstrating clinical improvement have not shown any associated changes in IgE or IgG levels [34]. Although these therapies are attractive because they are less invasive and have a lower risk of systemic reactions, further research is required to assess the mechanisms and to optimize treatment regimes. Still less is known about the underlying mechanisms of intranasal and oral immunotherapy in humans.

2.3 Recent Developments in Existing Techniques for Allergen Vaccination

Recombinant DNA technology has provided the basis for large scale production of well defined purified allergens. This has also enabled identification of proteins and epitopes with highest allergenicity, and facilitated the development of peptide vaccines (below). Standardisation of existing extracts and administration protocols has also lead to improved safely.

3. NEW STRATEGIES FOR IMMUNOTHERAPY

3.1 Allergen Gene Vaccination

The administration of plasmid DNA (pDNA) encoding allergic epitopes has produced encouraging results in animals [35-37]. Because of fewer side effects these strategies are attractive for human application. In mice, immunisation with allergen-*gene* vaccines produces Th1 responses, whereas allergen-*protein* vaccines favour Th2 responses [38]. The main reason for the strong "Th1 biasing effect" of gene vaccination is the presence of highly Th-1 immunostimulatory bacterial CpG motifs in the plasmid vectors (reviewed by Spielberg [38]). This is discussed further below. Parenteral gene vaccinations have been administered both subcutaneously and intramuscularly, but the subcutaneous route appears more efficient in evoking a Th1 response [38] because of the greater number of antigen presenting cells in cutaneous tissues. Allergen gene-vaccination via the oral route also appears effective and has obvious advantages, particularly in paediatric populations. In animals, the oral administration of peanut allergen-genes has already been shown to modify immune responses to this allergen [39]. Although these strategies may be very attractive, the regulation of allergen gene transcription remains a concern. In one recent study (in mice), latex gene vaccination (Hev b1) resulted in widespread expression of transcripts throughout many lymphoid and non-lymphoid tissues [40]. As many allergens have potentially toxic characteristics, levels of expression need to be regulated. This is likely to delay human trials.

3.2 CpG Motifs as Immunostimulants in Allergen Vaccination

Conjugation of allergens to immunostimulatory bacterial DNA is a new promising development in allergen-specific vaccination. A number of strategies have been employed including conjugation of the allergenic proteins to adjuvant bacterial oligonucleotides, or incorporation of allergen encoding cDNA which will be transcribed by the host (reviewed by [41, 42]). These techniques are more effective and better tolerated than conventional allergen-vaccination. Conjugation renders allergen proteins less anaphylactogenic. Alternatively, allergen transcribed from cDNA is processed by intracellular pathways of CD8+ T cells, and is also unlikely to induce IgE mediated reactions. Preliminary studies indicate that a combination of CpG + DNA vaccination may be more effective in antagonising Th2 responses than the combination of CpG + protein immunisation [43].

The use of bacteria as adjuvants was reported in the 1960's, and proved to be effective although the reasons were then uncertain [44]. It is now evident that this was probably due to bacterial DNA which contains repeated immunostimulatory sequences (ISS) of unmethylated cytosine and guanosine (CpG motifs). These motifs (which are conserved among bacteria) evoke an efficient innate immune response as part of an important evolutionary defence mechanism. CpG motifs activate antigen presenting cells (especially dendritic cells) and natural killer cells, and promote the production of pro-Th1 signaling in the form of IFNα, IFNβ, TNFα, IL-12 and IL-18. This has been shown to enhance Th1 maturation and inhibit IgE production, although other mechanisms including IL-10 and CD8+ T cells may be involved. It was proposed that these adjuvants could suppress Th2 responses to specific allergens. This has been demonstrated in animal models [45], and does appear to be sustained. In mice, the administration of ISS oligodeoxynucleotides (ISS-ODN) coupled to rag weed allergen (Amb a 1) induced a Th1 biased (IFNγ) response to Amb a 1, with concurrent IgE suppression [45]. Although, it appears that this strategy will be more effective in down-regulating developing rather than established immune responses [45], these techniques are likely to proceed to human trials. Again, the possible long term effects are not known. As with other forms of Th1 simulation, there have been concerns about Th1 mediated complications including the production of endogenous complement fixing IgG, and associated disease.

3.3 Modified Allergens

Molecular technology has also allowed the generation of modified allergens (without disulfide bonds) that do not have tertiary structure. These have maintained immunogenicity (T cell stimulation), but significantly reduced allergenicity (IgE binding) with fewer side effects. A number of these allergens are under investigation in humans.

3.4 Allergen-Peptide Immunotherapy

One main problem of immunotherapy is adverse side effects especially anaphylaxis to whole native allergen, and for this reason it has not been advocated for treatment of food allergies, especially peanuts. Further, the lack of standardised reagents has also limited the application of this approach. Modern DNA technology and protein chemistry can overcome many of these problems. Many allergens, including those of peanut, bee venom, pollen, mites and animal dander have been cloned, sequenced, expressed and epitope mapped. Recombinant forms of allergens are becoming more widely used for skin prick testing and intradermal injection and are increasingly considered the best source for allergen immunotherapy [46,47]. Furthermore, the native sequence and structure can be manipulated to generate hypoallergenic mutants that no longer bind IgE but retain T cell epitopes, ie loss of allergenicity with maintenance of immunogenicity. A number of methodologies are being considered to generate hypoallergenic forms of allergens and these are discussed below.

Site-Directed Mutagenesis

Modern DNA technology allows the manipulation of the nucleotide coding sequence and therefore the resulting protein. Typically, substitutions and deletions of nucleotide bases are used to change the cDNA encoding an allergen leading to an altered amino acid sequence upon production of the recombinant protein. This modifies the tertiary structure and thereby the functional properties of the protein. Loss of native tertiary structure has a central role in loss of allergenicity as IgE epitopes are often discontinuous, being dependent on the juxtaposition in space of different parts of the molecule. In contrast, T cell epitopes being composed of a discrete string of amino acids, are generally not affected by changes in structure. There are a number of examples of the use of this technology. A Der f2 mutant (C8/119S) that has lost an intramolecular disulphide bond leading to structural changes no longer binds IgE from patients serum and instead induces a strong Th1 (IFNγ) response from the blood of atopic donors [48]. More recently three major peanut allergens (Ara h1, h2 and h3) were cloned, characterised and subjected to site-directed mutagenesis [49]. Amino acids critical to the IgE binding epitope of these allergens were identified and targeted for site-directed mutagenesis. The mutants were generally poor competitors for binding of peanut-specific IgE compared to the wild type and binding by IgE from patient serum was reduced. The mutant allergens also retained the ability to stimulate proliferation by most patients and they, or their derivatives, may be suitable candidates for use in immunotherapy.

Short Peptide Therapy

Hypoallergenic peptide fragments (approximately 20 amino acids) that retain the ability to stimulate a T cell response are also being investigated for usefulness in immunotherapy. These fragments are generally produced using commercially available peptide synthesisers using now standard techniques. Preliminary analysis of peptides confirmed that priming with an appropriate peptide or peptide pool could tolerise for subsequent challenge with the whole antigen. This was demonstrated using the immunodominant T cell epitope of Der p1 [50] which also limited production of all T cell cytokines but had no effect on IgE production [51]. Two peptides (27 amino acids in length) derived from chain 1 of the major cat allergen Fel d1 (IPC-1 and IPC-2) corresponding to amino acids 7 - 33 and 29 – 55, respectively were as effective as entire chain 1 in limiting (prophylactically and therapeutically) the response by mice to Fel d1 [52]. These peptides were administered intranasally or sub-cutaneously, respectively, making them candidates for immunotherapy in humans. As yet there have been only limited human studies of their efficacy and safety. Although Der p1 peptides have not been trialed in humans, Fel d1 peptide therapy has proved of limited success. Reduced allergy scores (nasal and lungs) were recorded 6 weeks after the completion of treatment (s.c. injection of IPC-1 and 2 each week for four weeks) [53] and IL-4 production was significantly lower in the treatment compared to placebo group but there was no change in IE, IgG or IFNγ [54]. These effects were not seen with treatment lower than 750 g/dose [53-55]. Strikingly, nearly 70% of subjects in the tratment groups had adverse reactgions, predominantly a late asthmatic reaction characterised by allergic rhinitis and asthma symptoms. There was no immediate or late phase skin reactivity. In fact the response to shorter peptide fragments of Fel d1 (16/17 amino acids) has been used to characterise this late asthmatic reaction [56]. Intradermal injection of pooled Fel d1 fragments did not induce an immediate reaction but in a subset of donors there was delayed reaction characterised by reduced FEV_1. This late asthmatic reaction had some association with the HLA genotype of the subjects and supports a central role for T cells in the late asthmatic response.

Long Peptide Therapy

In addition to the reported adverse reactions to short peptides, one of the major shortcomings of short peptide immunotherapy is the requirement for information about the subject's T cell epitope usage. These will vary with the individual's tissue type (HLA genotype) and the short peptide pool may need to be tailor made to suit the donor.

Long peptide technology, as the name suggests, utilises long peptides that span the entire sequence of the protein thereby ensuring all T cell epitopes are available in the absence of IgE binding. Knowledge of all the T cell epitopes within an allergen aids in the design of the fragments but details of the subject's HLA genotype are not required because all epitopes are present. These fragments can be generated as described for short fragments or by PCR using the wild type cDNA as the template then cloning into a plasmid for expression by *E. coli* and purification of the protein. The two best examples being considered for use in immunotherapy are PLA_2 and Bet v1.

PLA_2

Three overlapping fragments of PLA_2 have been generated corresponding to amino acids 1 – 60, 47 – 99 and 90 – 134. These fragments have been used successfully (intranasal and intra-peritoneal administration) in mice sensitised to PLA_2 in alum leading to reduced specific IgE and IgG1, increased IgG2a (Th1 response in mice) and decreased IL-4:IFNγ [57,58]. Thus a shift from Th2 to Th1 responsiveness was induced. Intra-nasal or intra-peritoneal administration of the fragments also prevented anaphylaxis. Further elaboration of the immunological effect of these fragments was provided by the ability to transfer specific T cell tolerance to naïve mice using CD4+ T cells [59]. Although further phenotyping was not conducted this observation suggests that an anergic or regulatory population was produced during successful immunotherapy with long peptide fragments paralleling the observations made using current therapy modalities in humans.

Preliminary data is available on the potential usefulness of these molecules for treating humans. Long PLA_2 fragments, unlike shorter overlapping peptides, induced vigorous T cell proliferative responses from all PLA_2 sensitised allergic patients undergoing conventional venom immunotherapy, and did not bind IgE or trigger skin reactions on intradermal injection [60, 61].

Bet v1

In contrast to PLA_2 two non-overlapping long fragments of Bet v1 have been engineered; an N-terminal fragment corresponding to amino acids 1 – 74 and a C-terminal fragment that corresponds to amino acids 75 – 160. The breakpoint has been designed to correspond to a point outside all known epitopes. Neither fragment could bind IgE but the C-terminal fragment induced greater proliferation and favoured a potent IFNγ response whereas the N-terminal favoured IL-4, IL-5 and IL-13 production. Administration of the fragments to animals led to the production of IgG that inhibited IgE binding by serum from allergic patients [62]. Thus these Bet v1 fragments, especially fragment C, have the properties desired in a candidate for successful immunotherapy. These fragments have also been tested by skin prick test and intradermal injection. Lower reactivity than wild type Bet v1 was observed with greater than 100 fold more fragment needed to induce immediate type skin reactions [63]. A comparison of wild type Bet v1 and peptide derivatives has been undertaken in a human skin chamber model using birch pollen sensitised subjects. Chamber fluid was collected 2 and 8 hours after administering the peptides or wild type Bet v1 and examined for various mediators. Although the levels of eotaxin and the number of eosinophils recruited to the fluid were similar for the two groups, cellular activation was reduced in the peptide treated group; lower levels of histamine, granulocyte/macrophage-colony stimulating factor (GM-CSF) and eosinophil cationic protein (ECP) were recorded and eosinophil expression of CD69 (a common activation marker) was diminished [64]. Animal models indicate that peptide pools can be used, prophylactically and therapeutically, for alleviating the allergic response to Bet v1. Mucosal administration (intranasal) of Bet v1 peptides reduced specific responses by B and T lymphocytes both *in vivo* and *in vitro*, limited eosinophil infiltration of the airways and decreased airways hyperresponsiveness in subsequently sensitised animals. This response was comparable to the prophylactic effect of the administration of intact wild type Bet v1 prior to sensitisation of the animals [65].

Thus, long peptide fragments have a promising future in immunotherapy although assessment of the long-term benefits is essential especially as PLA_2-specific IgE gradually increased in the long peptide treated mice in the studies described above. However, reversibility provides further support that T cell anergy rather than deletion is central to immunotherapy success.

Other Protein Manipulations

Structural mimotopes are discovered using random phage display peptide libraries. This technology has been used to yield IgG that blocks rather than enhances IgE binding/cross-linking which otherwise is not predictable. By panning with a murine antibody that enhances IgE binding to Bet v1 and using the mimotope to immunise mice, the investigators generated IgG that also enhanced this reaction. Conversely, by panning for mimotopes recognised by Bet v1-specific IgE from a birch pollen allergic patient and immunising mice with the identified mimotope, resulted in IgG that blocked IgE binding *in vitro* [66].

Other modifications of protein allergens have been considered. Addition of a maleyl group to a protein targets the scavenger receptors on macrophages which is postulated to bias towards Th1 responses. This has been attempted with tropomysin, the major shrimp allergen. In a mouse model administration of maleylated tropomysin led to decreased IL-4 and increased IFNγ production compared to the native antigen [67]. Prophylactic and therapeutic oral administration of cholera toxin B (CTB) coupled with ovalbumin reduced specific IgE levels in plasma from appropriately sensitised mice despite CTB being considered to down-regulate Th1 rather than Th2 responses [68].

3.5 Anti-IgE Antibodies

As a central mediator in allergic disease, IgE is a logical therapeutic target. There has been intense interest in the development of therapeutic antibodies that can bind IgE without inducing histamine release. There are a number of strategies which have been investigated including:

Passive:

i) Allergen-specific passive immunisation with (anti-idiotype) IgG antibodies to variable regions of IgE,

ii) Nonspecific passive immunisation with antibodies to constant (Fcε regions of IgE (targeting all IgE),

Active:

iii) Vaccination to induce active production of anti-idiotype antibodies to target allergen-specific IgE,

iv) Vaccination to induce active antibodies to all IgE (Fcε),

v) The use of allergen-antibody immune complexes to down regulate production of specific IgE.

The principal challenge for all these techniques has been to bind and inhibit IgE activity without inducing IgE cross linking and mast cell degranulation. The use of antibodies to Fcε for passive immunisation is the most developed technique (already used in human clinical trials) but does have some limitations. While this therapy achieves dramatic (non-specific) reductions in total serum IgE levels and clinical improvement (summarised briefly below) it requires repeated administration of high doses of monoclonal anti-Fcε antibodies, with obvious cost. More recently, techniques for safely inducing sustained endogenous production of anti-Fcε antibodies have been explored. [69], although these studies are still limited to animal models. Strategies to selectively inhibit the activity of allergen-specific IgE are also not yet well developed in humans. Because these techniques rely on binding at the variable allergen recognition regions of IgE, these anti-idiotype antibodies cannot prevent binding of IgE to mast cells. The risks of inducing IgE crosslinking and mast cell degranulation [70] are a major limitation. The development of humanised mouse IgG4 antibodies (which are less likely to crosslink

IgE) may overcome this, and vaccine strategies to induce naturally occurring anti-idiotype IgG4 responses may have more sustained benefits. However, because of the demonstrated greater safety of anti-Fcε antibodies in passive vaccination, and remaining concerns that antibodies to other portions of IgE may be anaphylactogenic, most techniques for active vaccination are focused on inducing antibodies to Fcε domains [69]. In animals there have been successful attempts to neutralise the effects of IgE by vaccination with Cε3 and Cε4 epitopes.

Antibodies to IgE Fcε

The identification of monoclonal antibodies (mAb) to human IgE lead to the development of humanised non-anaphylactogenic antibodies to IgE. These have shown beneficial effects in human trials (reviewed in [71-74]). Although the effects of these antibodies are not allergen-specific, they are relevant to this discussion because of the potential benefits and complimentary effects in the treatment of many specific allergies.

These antibodies (CGP 51909 and rhu mAb E25) bind to the Fc (Cε3) domain of IgE that normally binds to mast cells via the high affinity IgE receptor FcεR1. This achieves a very efficient reduction in the free serum IgE (by more than 90% in some studies [75], and prevents binding to mast cells (and basophils) and the downstream inflammatory effects. The resulting immune complexes (with E25) are very small [76] and do not activate complement or accumulate in any body organs [77]. As the B cell binding domain on IgE is distinct from the Cε3 mast cell binding region, these mAb can still recognise IgE on B cells and suppress IgE production. The immunological benefits of anti-Cε3 mAb do not appear to be limited to reducing the acute phase response [78]. Anti-IgE mAb also prevents IgE binding to FcεRII (CD23) receptors on monocytes, dendritic cells, epithelial cells and possibly eosinophils (reviewed by [74]). These changes have been associated with clinical improvement and a significant reduction in medication requirements for both asthma [75, 79-81] and allergic rhinitis [82, 83]. It is worth noting that immunological parameters improved more consistently than clinical symptoms, and few patients show complete resolution of their symptoms. The reason for heterogeneity in the clinical response despite immunologial improvement is unknown. It is likely that other IgE independent pathways are involved in disease expression, as noted in IgE/B cell deficient mice [84, 85]. While systemic administration of anti- Cε3 mAb reduces circulating levels of IgE, there may be little effect in the end-organ tissues where disease is expressed. However, efforts to deliver anti-Cε3 mAb topically to mucosal sites of atopic inflammation have been ineffective so far [86].

As administration of anti-Cε3 mAb is a form of "passive" immunisation, the clinical and immunological effects of anti-IgE (rhu mAb E25) are also not sustained after therapy is discontinued. The development of newer strategies to promote endogenous production of anti-(Cε3) IgE mAb by "active immunisation" may be a way of achieving better end-organ effects and a more sustained effects. This has been recently achieved in animals [69]. Oral administration of bacteriophage expressing homologous IgE structures (Cε3 and Cε4) can induce systemic anti-IgE responses to. The potential of this strategy in humans is uncertain.

Although anti-Cε3 mAb have been given safely and effectively in humans, the long term consequences are unknown. Potential antigenicity of murine derived antibodies has been minimised by removing nonessential murine residues [87], with fewer than 5% mouse sequences in the remaining products (CGP51901 and E25). To date there are no reported cases of sensitisation to the remaining murine residues. Initial concerns that inhibiting IgE may compromise the host response to parasites also appear unfounded. In fact the efficient clearance of parasites by infected animals treated with anti-IgE mAb [88, 89] has challenged the previously held assumptions about the role of IgE in parasitic disease.

These antibodies will be soon available for clinical use. It is important that there are clear guidelines and indications for use. At this stage anti-(Cε3) IgE mAb appear inappropriate as monotherapy for asthma. As demonstrated in clinical studies [90] these antibodies have a steroid sparing effect in patients with moderate to severe asthma and may be useful in this context. This non-allergen specific therapy may have a role in patients with multiple sensitivities for whom vaccination for all specific allergens is not practical. IgE monoclonal antibodies may also enable a wider application of allergen-specific immunotherapy. Used in conjunction with allergen vaccination, anti-(Cε3) IgE mAb may reduce the risk of systemic reactions and possibly enhance clinical responses. This requires further investigation.

Anti-Idiotype Antibodies

Antibodies to the variable antigen-recognition region of IgE have been largely discussed above. At present these strategies have limited application in humans.

Antibody-Allergen Immune Complexes

The administration of autologous antibody-allergen complexes was used as a treatment strategy in the early 1990's and has shown clinical benefits in allergic patients [91-95]. This is highly allergen specific, and appears to mediate a reduction in the level of circulating antibodies with specificity for the administered allergen [96, 97], with reduction in both IgG and IgE antibodies. Furthermore, these antibody-allergen complexes appear to boost the production of anti-idiotypic antibodies, which may be one mechanism of their action. Research in this area has been overshadowed by the implementation of human trials with anti-(Cε3) IgE mAb.

3.6 Molecular Strategies (Cytokines) for Immune Modulation

The following methods of immunotherapy are not antigen-specific but offer alternative targets for immunotherapy, especially for the multi-sensitised patient. Subcutaneous administration of recombinant native human IL-12 has been trialed clinically with mild allergic asthmatic patients [98]. Eosinophil numbers in blood and sputum were significantly reduced compared to placebo but differences in

histamine-induced airways-hyper-responsiveness and the late asthmatic reaction to inhaled allergen were not significant. Similarly a single intravenous infusion of monoclonal anti-IL-5 prevented allergen-induced eosinophilia (sputum and blood) but did not affect airways hyper-responsiveness or the late asthmatic reaction [99]. These studies provide a cautionary tale: effectively targeting one component of the allergic response may not alleviate clinical symptoms. Obviously we have a long way to go before we fully understand the role of individual components, especially eosinophils, in the disease process. This is amplified because of the complexity, interplay and apparent redundancy within the immune system.

The development of cytokine variants that have improved or altered functional properties is another area of investigation. This requires an intimate and detailed knowledge of the structure of ligand and receptor. Functional variants can be generated in two ways. Site–directed mutagenesis, as discussed above, can be used to modify the cytokine's amino acid sequence so that interaction with the receptor is altered. For example, antagonistic IL-4 (hIL-4Y124D) has been designed that inhibits IL-4 and IL-13 induced IgE synthesis [100]. An alternative approach is molecular breeding/DNA shuffling which can yield novel variants of cytokines especially as it bypasses the need for assumptions at the design stage [101]. This technology is, however, in its infancy.

Another target for novel strategies is FcεRI expressed on mast cells and basophils. The cross-linking of high affinity IgE receptors by IgE binding of multi-valent allergen triggers the release of biological mediators. Preventing degranulation will limit the early response. The intracellular component of FcεRI contains an immunoreceptor tyrosine based activation motif (ITAM). This introduces the possibility that activity of this receptor can be negatively regulated by co-aggregation of FcεRI with an ITIM (I= inhibitory) containing receptor. An example of an ITIM containing receptor expressed by mast cells that co-aggregates with and negatively regulates FcεRI signal transduction under physiological conditions is FcγRIIb (CD32b), the low affinity IgG receptor (reviewed by [102]). Importantly, IgE induced release of biological mediators by mast cells and basophils can be inhibited by cross-linking FcεRI and FcγRIIb under physiological conditions. Co-aggregation of these two receptors in mouse mast cells reduces the secretion of serotonin and TNFα [103]. Moreover, mice deficient in FcγRIIb have enhanced IgE-mediated anaphylactic responses indicating that FcγRIIb has a physiological role in regulating the response triggered via the high affinity IgE receptor [104]. Theoretically, specific IgG or allergen complexes can be engineered to enhance the inhibitory interaction of FcεRI and FcγRIIb and limit degranulation of mast cells and basophils.

4. PROGESS IN IMMUNOTHERAPY FOR TREATMENT OF SPECIFIC ALLERGIES

4.1 Inhalant Allergies

While inhalant immunotherapy is well established a number of new strategies may improve available therapy. Peptide immunotherapy and DNA vaccines may offer safer alternatives to protein extracts. Extracts more relevant to different geographical locations also need to be developed. Better mucosal vaccines may also become more important, particularly for primary prevention (see below).

4.2 Insect Venom Allergies

Whereas traditional immunotherapy for bee venom uses whole venom, the identification of short peptides of the immunodominant allergen PLA2 (phospholipase A2) holds future promise as an effective but safer alternative (see peptide vaccination). In sensitised mice the administration of long overlapping peptides spanning the whole PLA2 molecule has resulted in successful modulation of cellular responses and also fully protected from anaphylaxis [58] Similar strategies are being developed for humans.

4.3 Food Allergy

Although many food allergies are transient, there is still an urgent need for new therapies for persistent life threatening allergies to foods such as peanuts. Immunotherapy for food allergy is not generally accepted because of the high risk of serious, potentially life threatening IgE mediated systemic reactions. Although there are isolated reports of desensitisation for peanut allergies [105], serious side effects are common, there are no standardised protocols and this is considered too dangerous by most.

More recently, site-directed mutagenesis of major peanut allergens has rendered peanut allergens more "hyoallergenic" [49]. These modified allergens are less likely to produce systemic reactions but remain immunogenic, and may lead to safer immunotherapeutic options in the future. Anti-IgE mAb may be also be of theoretical benefit (in conjunction with allergen-specific vaccines) by reducing serious adverse reactions to food allergens. The anticipated development of allergen peptide immunotherapy, and allergen-gene vaccination may offer future hope. Small peptides or transcribed allergen genes will theoretically induce tolerance at a cellular level without evoking IgE reactions. In animals it has been possible to induce systemic tolerance to a protein using a single epitope [50]. There has unfortunately been slow progress for food allergy in humans.

4.4 Drug and Latex Allergy

Rush desensitisation protocols are used widely, particularly for antibiotics when the drug is highly indicated for a hypersensitive patients. This remains a dangerous procedure and unfortunately there are few more definitive approaches on the horizon. There may be a role for non-specific IgE inhibition anti-($C_\varepsilon 3$) IgE mAb prior to rush protocols. To our knowledge, this has not been examined.

Extracts for latex desensitisation are now available, and can induce tolerance in sensitised individuals [106, 107]. However, systemic side-effects remain a serious concern. In 1997, Slater *et al.* [35, 108] reported favourable response in latex sensitised mice which were vaccinated with plasmid

DNA encoding latex allergen Hev b 5, with a 23% drop in the levels of allergen-specific IgE after 10 days. This form of allergen-gene vaccination offers new hope for may allergies with no conventional immunomodulatory therapy.

5. ALLERGEN VACCINATION FOR PRIMARY PREVENTION

With the escalating incidence of allergic disease there is a growing need to develop strategies to prevent the development of Th2 responses. The use of allergen vaccines has been proposed as one method of primary prevention [109]. While the therapeutic benefits of "allergen vaccines" in existing disease are well established, the role in disease prevention is still largely theoretical in humans. Potential strategies involve utilising and enhancing the natural processes which in most cases efficiently terminate IgE responses to allergens in infancy. Accordingly, vaccines for primary prevention would need to be administered in early infancy, when immune responses are still "plastic" and not "committed". This is not without concerns (discussed further below). In murine systems neonatal administration of allergen can inhibit the development of Th2 type airways disease, but the dose and delivery method appear crucial [110].

The enteric mucosal immune system plays an extremely efficient and pivotal role in the development of tolerance. Repeated exposure to allergen through the gastrointestinal tract during this period of life leads to the development of tolerance, even in highly atopic individuals (reviewed in [109]). It is proposed that exposure to aeroallergens through this route may promote the local (IgA) immune responses which promote persistent systemic tolerance, preventing the emergence of pathogenic Th2 responsive memory T cells. In animal models, early antigen feeding induces tolerance possibly mediated by allergen-specific suppressive γδ+ CD8+ T cells [111] as well as the non-specific effects of TGFβ. It now also appears that a population of CD25+ CD4+ T regulatory cells also have an important role in regulating systemic tolerance [112, 113]. A number of studies are currently addressing the effects of these strategies in humans, including the effects of intranasal administration of allergen, which may theoretically have similar benefits. Parenteral administration of allergen with an appropriate Th1 inducing adjuvant is also being considered for promoting selective Th1 responses during immune development. It is possible that the effects described in adults with established disease may be more effective in infants without established patterns of T cell response.

The main concerns about allergen vaccination in infants, particularly while the precise mechanism of action are uncertain, are potential unforeseen consequences on other aspects of natural immune development. It is possible that bystander effects could mediate altered patterns to other antigens. The parenteral administration of Th1-enhancing adjuvants raises theoretical concerns about Th1 mediated autoimmune reactivity. While there is a very high risk of atopic sensitisation (almost 40% in industrialised countries) [114] many will only develop mild disease, and it may be more desirable to target invasive prevention strategies to infants who are most likely to develop severe disease. It is currently not possible to accurately predict clinical outcomes. Although allergen vaccination may become a useful strategy, at this stage it is more critical to determine the underlying reasons for the dramatic increase in allergic disease, and the mechanisms of immune dysregulation in this group.

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